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The study of delivery medicine using ends device: a bromhexine case study

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ABSTRACT: Electronic Nicotine Delivery System (ENDS) has been used over the last decade to substitute conventional cigarettes. Active pharmaceutical ingredient (API) delivery using these devices has been reported in the literature, mainly for bronchodilators and nicotine; however, mucolytic agents have not previously been evaluated through this route, and many puffs were required to achieve the therapeutic dose in existing formulations. This study aimed to deliver and measure bromhexine hydrochloride (BRM) as API via inhalation using an e-liquid formulation. The e-liquid was obtained by trapping the aerosol and measuring the concentration using high-performance liquid chromatography-mass spectrometry (HPLC-MS) in multiple reaction monitoring (MRM) modes. Our study demonstrated that five puffs accumulated 3.3156 ng/mL of BRM, and the efficiency of the ENDS device was calculated to be 99.8%. In addition, computational simulations were performed, resulting in a binding affinity of BRM of -7.3 kcal.mol-1 compared to salbutamol with -8.1 kcal.mol-1. Nevertheless, BRM has a hydrogen bond between the amine group and Ser204, Pi-Alkyl bonds with Ala200 and Val114, also alkyl interactions with Phe289 and Phe290. However, the BRM interaction with the protein does not form a stable complex in the dynamics simulation, but it may act as an allosteric modulator or partial agonist. In conclusion, the results of this study corroborate previous results that ENDS technology may have wide innovations for inhalation medicines.

KEYWORDS: Bromhexine hydrochloride; computational simulation; electronic nicotine delivery system; inhalation delivery.

▪ INTRODUCTION

Inhaled active pharmaceutical ingredients (API) administration is one of the non-invasive drug delivery methods that is highly desirable for the treatment of respiratory disorders due to its low systemic adverse effects and efficient pulmonary targeting [1],[2] However, the patient's body, the device, and the formulation all need to work in synergy to achieve optimal aerosol delivery These devices should be capable of producing appropriately sized particles, be simple to use, affordable, portable, and, most importantly, "personalized" to meet individual patient needs. Alternative aerosol-generating drug delivery systems have been widely investigated in recent years [3],[4].

Electronic Nicotine Delivery Systems (ENDS) have established themselves as a smoking cessation aid and are often perceived as a less harmful alternative to conventional cigarettes [5]. The number of people using ENDS is expected to rise by about 90 million in 2023. Over the past decade, ENDS technology has evolved into advanced generations capable of temperature regulation, low-resistance (low-ohm) heating elements, and adjustable airflow systems [6], [7]. ENDS-generated aerosols are complex mixtures of droplets and vapors that undergo dynamic physicochemical processes such as evaporation, condensation, and coagulation during transport through the respiratory tract [8]. These devices can generate submicron particles and deliver relatively consistent doses of active substances per puff. The potential of ENDS as an alternative drug delivery platform has therefore attracted increasing scientific interest. Modern ENDS are also considered potentially more acceptable to patients, which may improve adherence [4].

Recent studies suggest that the familiar design and user-friendly operation of ENDS may enhance patient adherence compared to conventional inhalers, which often require precise coordination and inhalation

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technique for effective drug delivery [9]. The ability of ENDS to generate submicron aerosols enables efficient deposition of active substances in the lower respiratory tract, potentially improving therapeutic outcomes [10], [11]. Additionally, features such as adjustable airflow and temperature control allow for more individualized dosing strategies, thereby enhancing usability and compliance [12],[13]. Furthermore, modeling studies indicate that ENDS constituents are primarily absorbed in the pulmonary region, where gas exchange with the bloodstream is most efficient, supporting their potential for systemic drug delivery. Moreover, compounds with lower volatility are mainly absorbed through droplet deposition, while higher-volatility compounds are absorbed through vapor-phase uptake, enabling relatively high overall retention of inhaled substances and suggesting suitability for a broad range of therapeutic formulations [14].

While these advantages position ENDS as promising alternatives to traditional inhalers, especially for individuals who struggle with inhaler technique, further research is needed to fully establish their safety and efficacy for delivering medications beyond nicotine, despite its controversy as an unintended illicit drug delivery system [15].

Several studies demonstrate the capability of ENDS to deliver active pharmaceutical ingredients (APIs). Herbert et al. used fluorine-18 for radiolabeling [16], while Khaleed et al. administered fluticasone propionate via ENDS [17]. Research also indicates that methamphetamine can remain stable in ENDS e-liquids at detectable concentrations. Additionally, cannabinoids and 3-4 methylenedioxymethamphetamine (MDMA) have been effectively delivered through ENDS [15]. For pulmonary applications, ENDS have been shown to deliver terbutaline with consistent respirable doses, where approximately 52 puffs achieved a dose equivalent to a single nebulization session. Similarly, medical-grade vaping devices support the stable delivery of salbutamol, highlighting their potential in respiratory drug delivery [18]. These studies focus on bronchodilators. Mucolytic agents such as BRM, despite their established role as COPD co-treatment, have not been evaluated for ENDS-based delivery. Given these findings, ENDS may have therapeutic potential for respiratory diseases such as Chronic Obstructive Pulmonary Disease (COPD), where noxious particles, gases, and tobacco smoke contribute to progressive airflow limitation [15],[19].

Based on the Global Initiative for Chronic Obstructive Lung Disease Guideline, the initial maintenance treatment for COPD includes bronchodilators, for example Long-Acting β2 Agonist (LABA) and Long-Acting Muscarinic Antagonist (LAMA) or Short-Acting β2 Agonist (SABA) like Salbutamol and Short-Acting Muscarinic Antagonist (SAMA) as a reliever [19]. As co-treatment, mucolytics can be used in selected patients, particularly those with chronic bronchitis, productive cough, or frequent exacerbations [20]. Thiol-based mucolytics like erdosteine, N-acetylcysteine (NAC), and carbocysteine are commonly used, particularly in patients with moderate COPD (FEV1 50–79% predicted) [19],[21]-[23]. In addition, bromhexine hydrochloride (BRM) has also been evaluated in COPD. Prabhu Shankar et al. (2010) conducted a multicenter, double blind, randomized trial involving 426 patients with productive cough, including those experiencing acute exacerbations [24].

BRM is a mucolytic drug commonly administered orally. It has been shown to modify mucus properties and has demonstrated therapeutic benefits in various conditions, including xerostomia, nephropathy, asthma, and bronchitis [25]. BRM has been shown to change the sputum characteristics in vitro, but it has produced varying results in several clinical trials [25],[26]. Orally administered bromhexine is rapidly absorbed but undergoes extensive first-pass metabolism, resulting in low absolute bioavailability of approximately 20–27%. Lung tissue concentrations of bromhexine two hours post-dose were 1.5 to 3.2 times higher in bronchial tissues than in plasma, while pulmonary parenchyma concentrations were 3.4 to 5.9 times higher than plasma levels [27]. Although these pharmacokinetic properties are promising, the effectiveness and safety of inhaled BRM delivered via ENDS have not yet been extensively evaluated in clinical settings, and further studies are required to establish its clinical utility.

The urgency of this research stems from the growing need for alternative drug delivery systems that improve patient compliance and therapeutic outcomes. Despite the potential of ENDS technology for drug delivery, significant gaps remain in the literature. Most studies have focused on nicotine delivery, with limited exploration of therapeutic agents such as mucolytics via ENDS. Furthermore, integrated evaluations combining experimental quantification of drug delivery with computational modeling of molecular interactions and ADMET properties remain scarce. This study addresses these gaps by investigating the potential of ENDS to deliver BRM as an inhaled mucolytic therapy.

The novelty of this study lies in its integrated approach, combining experimental quantification of BRM delivery via ENDS using HPLC-MS analysis with computational molecular docking, molecular dynamics simulations, and ADMET predictions to evaluate BRM interactions with the β2-adrenergic receptor. This multidisciplinary framework provides both empirical evidence of drug delivery feasibility and mechanistic insight into receptor-level interactions, offering a comprehensive evaluation of ENDS-based BRM delivery potential.

This study aimed to investigate the feasibility of delivering free-base BRM via inhalation using e-liquid formulations aerosolized through ENDS technology as a mucolytic therapy. This objective was achieved through mass spectrometry analysis to confirm the presence of BRM and quantify its concentration under different experimental conditions. In addition, molecular docking simulations were conducted to compare the interaction of BRM with salbutamol, as a standard inhaled drug.

▪ MATERIALS AND METHODS

Materials

Bromhexine hydrochloride (BRM) (purity 99.83%) was purchased from Merck (Darmstadt, Germany). The solvents (propylene glycol and vegetable glycerin) used for the formulation of e-liquid were obtained as food-grade supplied by local suppliers. Hypergrade solvents (acetonitrile and methanol) for HPLC-MS analysis were purchased from Merck.

The instruments which used in this study are Waters Xevo-G2 XS QToF (Waters, Milford, Massachusetts, USA) equipped with C18 BEH column (2.1 × 50 mm, 1.7 µm) as the stationary phase was used for Multiple Reaction Monitoring (MRM) analysis. The acetonitrile (B) and ultrapure water (A) supplemented with 0.1% formic acid were used as the mobile phase. A gradient system starting from 5% B held for 1 minute, followed by increasing to 100% B in 12 minutes and holding for 3 minutes at 100%, and finally bringing back to the initial conditions in 3 minutes was applied.

Formulation of e-liquid

The e-liquid was prepared by accurately weighing 1 gram of BRM (98.7% purity) and dissolving it in a mixture of propylene glycol (PG) and vegetable glycerin (VG). The solvents were combined in a 1:1 (v/v) ratio, after which the active compound was gradually introduced under continuous stirring to ensure complete dissolution. The final solution volume was adjusted to 100 mL, corresponding to a concentration of 1% (w/v) active ingredient.

Preparation of standard bromhexine hydrochloride (BRM)

The stock solution of BRM 1000 µg/mL was prepared by weighing 5 mg of BRM and dissolving it in 5 mL of methanol (hypergrade) under gentle vortexing until complete dissolution was achieved. From this stock, two independent sets of standard BRM samples were freshly prepared, covering concentration ranges between 2 to 6 ng/mL and 10 µg/mL to 50 µg/mL, and were used to establish the standard calibration curve.

Sample preparation

The available ENDS devices (MOVI® MARK ZERO) in the market were purchased, including the cartridge (0.8 ohm). An aliquot of the formulated e-liquid (1 mL) was carefully dispensed into the cartridge. The generated aerosol from the ENDS device was collected using a fat-free cotton trapping system to capture the vaporized matrix. After five puffs, the BRM from entrapped aerosol residue was extracted from the matrix by dissolving the cotton in 2 mL of methanol (hypergrade) with the aid of sonication for 5 minutes to ensure complete leaching of the analyte. After sonication, the methanol was concentrated under vacuum to 1 mL.

Determination of the Limit of Detection (LoD) and the Limit of Quantification (LoQ)

The sensitivity of the HPLC-MS method was assessed by calculating the limit of Detection (LoD) and the limit of Quantification (LoQ). Both values were estimated from replicate analyses of low-concentration standards under the same experimental conditions. The LoD was calculated according to Equation (1).

$$LoD = 3.3\ x\ \left(\frac{SD\ of\ intercept}{slope}\right) \qquad \text{(Eq.1)}$$

While the LoQ was calculated according to equation (2)

$$LoQ = 10\ x\ \left(\frac{SD\ of\ intercept}{slope}\right) \qquad \text{(Eq.2)}$$

Computational Simulations

Molecular docking was performed using Autodock Vina run through the WSL system. The receptor protein target chosen was β-2 adrenergic receptor (β2AR), covering anti-inflammatory response at the bronchial smooth muscle when binding with its agonist. The full-length human β2AR structure was obtained from the Protein Data Bank (PDB) database (ID: 2RH1). The ligands BRM and salbutamol (as standard) were obtained from PubChem. Proteins were prepared by removing non-protein components (such as water, carbohydrates, lipids, etc.), and protein residues were inserted using a Modeler 10.5 based on their FASTA files. The polar hydrogens and charges were then added using AutodockTools. Similarly, the ligands were prepared using AutodockTools in flexible mode.

Molecular Dynamics (MD) simulation was conducted utilizing the open secure shell (SSH) connected to HPC BRIN Mahameru, as the national research and innovation agency in Indonesia.

The simulations were carried out on the B2AR protein, B2AR with BRM, and B2AR with SBM, and ran for 100 nanoseconds (ns) in duration with a trajectory graph spanning 105 picoseconds (ps). During the preparation, the docking results were taken, and the ionization state was adjusted using PROPKA calculation and pH conditions to 7.4 with the PDB2PQR website (https://server.poissonboltzmann.org/pdb2pqr). This study utilized the Gromacs MPI 2023.3 software with the Amber forcefield (ff99SB-ILDNP) owned in the system. The Protein topology file was made by the Gromacs Mpi 2023.3 program with SPC/E as water molecules. BRM and SBM were preprocessed by adding General AMBER Force Field 2 (GAFF2) from Antechamber using AmberTools23. The ligand topology files were aligned to adapt at the Gromacs environment using ACPYPE (AnterChamber Python Parser Interface). The parameter conditions of the system were regulated by equilibrating the temperature to 310.15 K and the pressure to 1 atm in the NVT and NPT scripts. The script was run for 1 ns with a velocity rescaling thermostat with a time constant of 0.1 ps and the pressure parameters with Parrinelo-Rahman barostat at a time constant of 2 ps. The Particle Mesh Ewald (PME) method was utilized to regulate the electrostatic interaction.

Absorption, Distribution, Metabolism, Excretion, Toxicity (ADMET) prediction

Absorption, Distribution, Metabolism, Excretion, Toxicity (ADMET) Prediction was conducted using machine learning https://biosig.lab.uq.edu.au/deeppk/prediction. This website was run by entering SMILES of SBM (CC(C)(C)NCC(C1=CC(=C(C=C1)O)CO)O) and BRM (CN(CC1=C(C(=CC(=C1)Br)Br)N)C2CCCCC2).

▪ RESULTS

Method Development and Validation Results

Since the ENDS relied on its properties to convert the e-liquid into an aerosol state, most of the experiments on measuring the concentration were conducted using the next generation impactor (NGI) [17]. The device was set to aerosolize the e-liquid, then trapped with cotton puffs and measured the concentration using high-performance mass spectrometry (HPLC). Two sets of standard bromhexine Hydrochloride (BRM) were prepared as the representative e-liquid. These two standards were chosen to accommodate the low dose of puffs and the large dose of puffs after the e-liquid of the pod was empty and needed to be refilled.

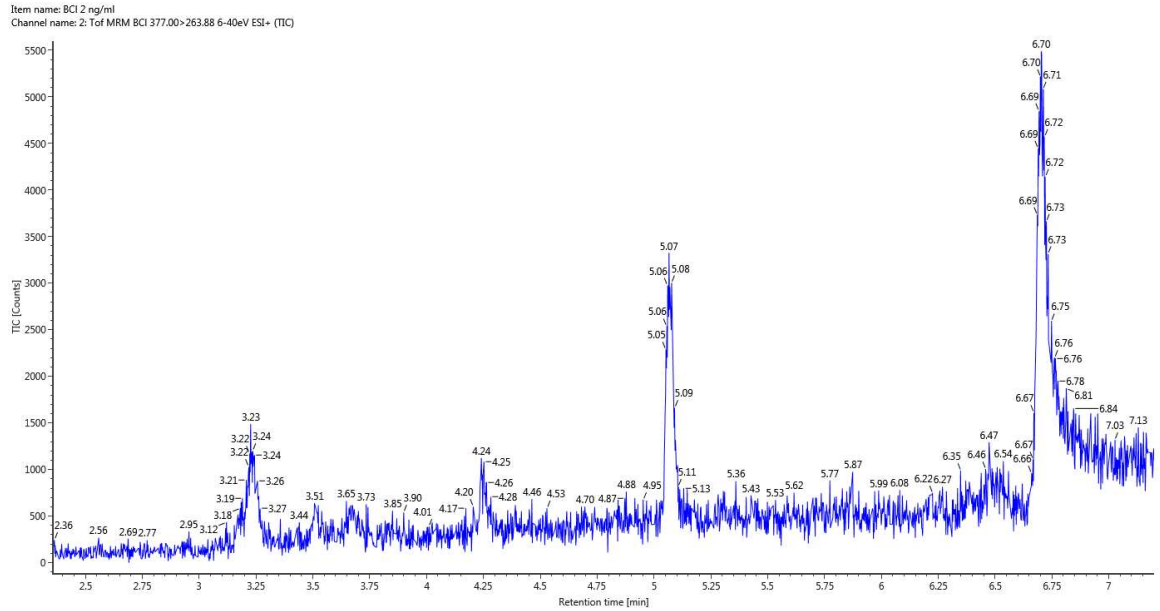


Figure 1. The example of the mass chromatogram of BRM (2 ng/mL) in MRM mode. The BRM was eluted at 5.06 minutes.

The quantification of BRM samples followed the method from El-Naem and Saleh (2021) by using multiple reaction monitoring techniques [28]. The first set of standards ranged from 2 to 6 ng/mL and had a correlation coefficient (r2) of 0.9931, as shown in Figure 2a. The limit of detection (LoD) is the lowest analyte concentration likely to be reliably distinguished from the limit of blank (LoB), and the limit of quantification (LoQ) is the lowest analyte concentration that can be quantitatively detected with a stated accuracy and precision [29], were tabulated in Table 1. The LoD and LoQ of the instrument were found to be 0.3014 ng/mL and 0.9138 for standard set 1, which is in agreement with the results from El-Naem and Saleh (2021) [28]. The initial measurement of the first sample with only one puff was unsuccessful (data not shown); this could have happened due to the concentration of the sample being lower than the LoD. Analysis of the sample after five puffs revealed that the content of the BRM was found to be 3.3156 ng/mL.

Table 1. The limit of detection (LoD) and limit of quantification of the two sets of standards.

No.	Standard set 1 (2 to 6 ng/mL)		Standard set 2 (10 to 50 µg/mL)	
	LoD (ng/mL)	LoQ (ng/mL)	LoD (µg/mL)	LoQ (µg/mL)
1	0.3015	0.9138	1.1718	3.5510

After successfully detecting the BRM in the trapped aerosols e-liquid, the calculations of the efficiency of the ENDS were performed by measuring the leftover e-liquid in the pod after the cartridge was empty. To do this experiment, the second set of BRM standards was established at a higher concentration i.e., 10 to 50 µg/mL, for comparison.

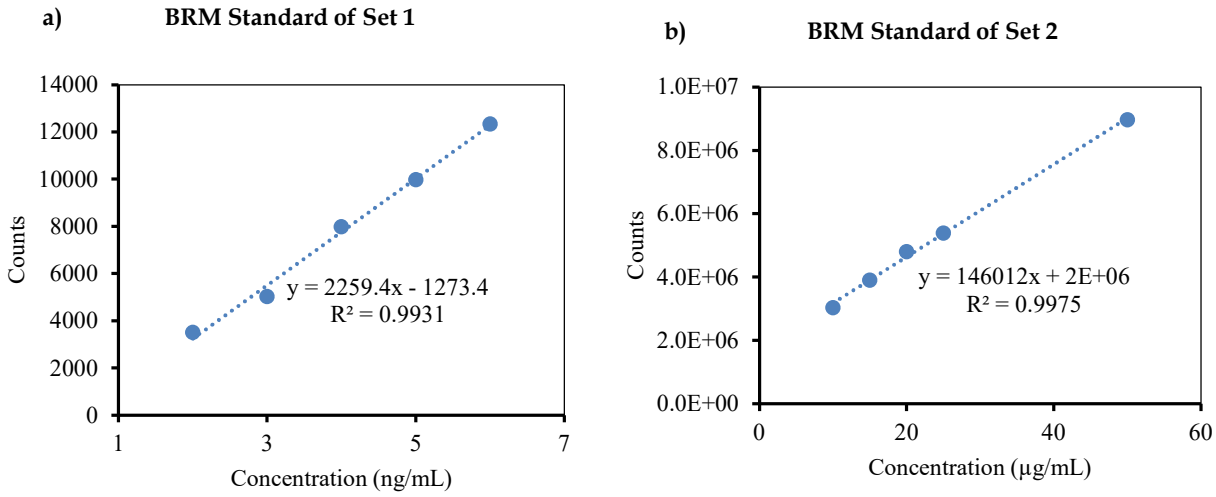


Figure 2. Calibration curve of a) the BRM standard samples of set 1 with the lowest concentrations ranged from 2 to 6 ng/mL and b) the BRM standard samples of set 1 with the highest concentrations ranged from 10 to 50 µg/mL.

After discharging the pod from the cartridge, preparing and measuring the sample, the residue of the BRM in the pod was calculated to be 19.3029 µg/mL. According to our experimental setup, the theoretical concentration in the BRM for each mL of e-liquid was 10.000 µg/mL. By applying the calculation of the BRM residue in the pod, the efficiency of the ENDS devices was calculated to be 99.80%.

Computational simulation analysis

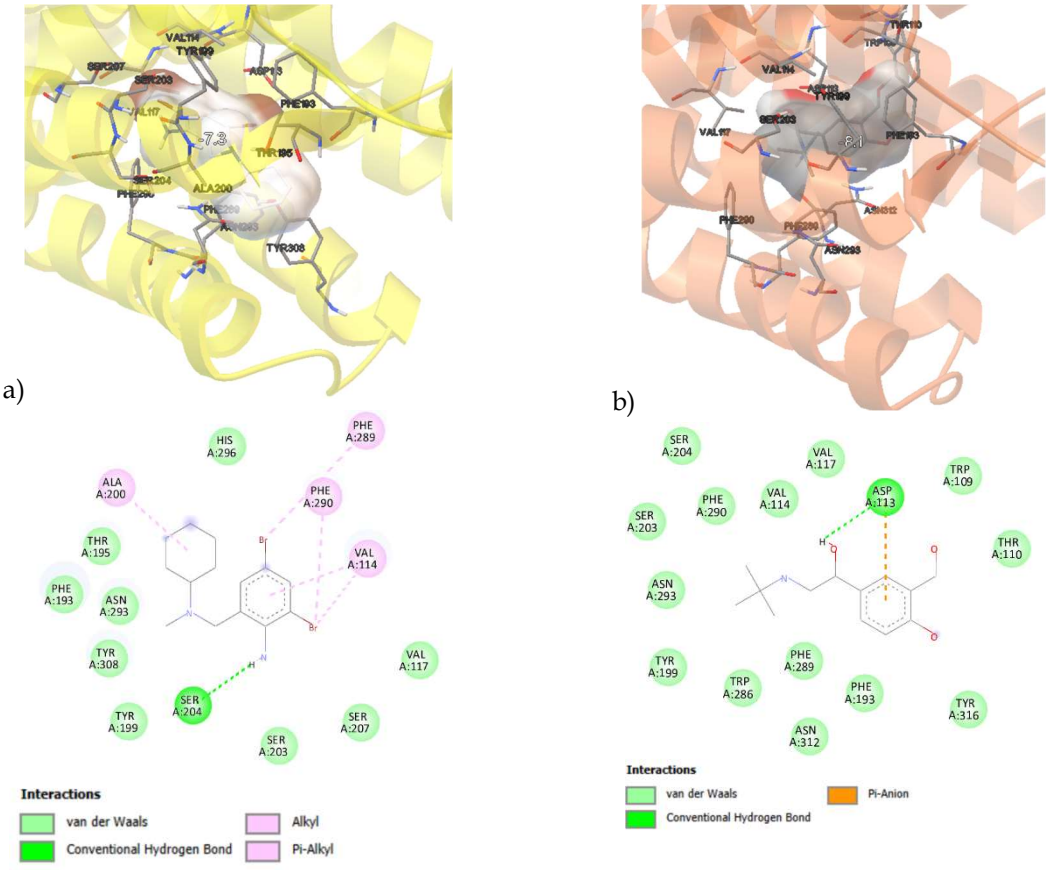


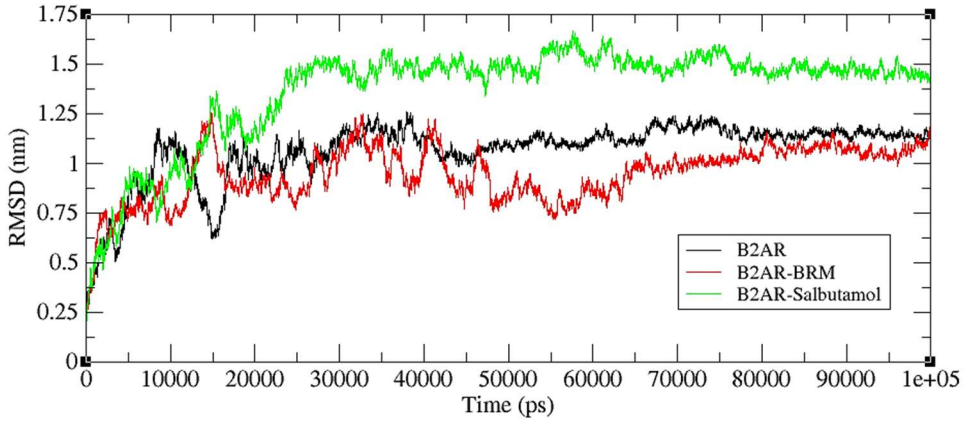
Figure 3. Molecular docking visualizations of a) BRM and b) Salbutamol.

Understanding the interaction between ligand and protein is necessary to determine the therapeutic effect of a drug on the body [30]. This simulation analysis was conducted using a molecular docking method targeting the active receptor site, which is approximately one-third of the protein sequence [31]. The interaction between β2AR and the ligand involves several amino acid residues that modulate the agonist effect. It has been reported that agonists tend to bind with amino acids, including Asp113 from TM3, Ser203, Ser204, and Ser 207 located on TM5, Asn293, Phe259, and Phe290 in TM6, and Tyr 308 in TM7. In addition, there are also interactions with His93, Trp109, Val114, Val117, Asn312, Ser316, Trp313, Asn312, and Tyr316 interact [31],[32].

The ligand position was determined based on the lowest affinity value with an RMSD value of about 2 Å at the active site coordinate. BRM has a lower affinity value than salbutamol, with a value of -7.3 kcal/mol compared to -8.1 kcal/mol. Although the resulting value is weaker than salbutamol, this value is not significantly different and is still classified as a good value for protein-ligand complexes [33]. As a standard, salbutamol interacts with the agonist active side of the receptor, where two Asp113 amino acid interactions occur in the form of hydrogen bonds that bind to the hydroxyl (-OH) group on the ethanolamine chain and pi-anion interaction with the benzene ring. Previous studies have reported several van der Waals (VDW) interactions on amino acid residues, including Trp109, Val114, Val117, Ser203, Ser204, Phe290, Asn293, Asn312, and Tyr316.

Compared with salbutamol, BRM also exhibits interactions with the active side of the receptor. As shown in Figure 3a, a hydrogen bond exists between the amine group and Ser204 in BRM, which exhibits the strongest affinity-forming bond. The two benzylamine rings have ionic interactions by forming Pi-Alkyl bonds with Ala200 and Val114, for which Val114 also has double ionic bonds with bromine groups. Alkyl interactions with Phe289 and Phe290 with bromine groups at different positions reinforced the ionic bonds. Noncovalent bonds are also supported by VDW interactions, strengthening the protein-ligand complex with Vall117, Phe193, Thr195, Ser203, Ser207, Asn293, His296, and Tyr308. The simulation demonstrated interactions between BRM and the active agonist side of β2AR, confirming the modulation of anti-inflammatory activity through the β2AR receptor on the bronchial membrane.

A



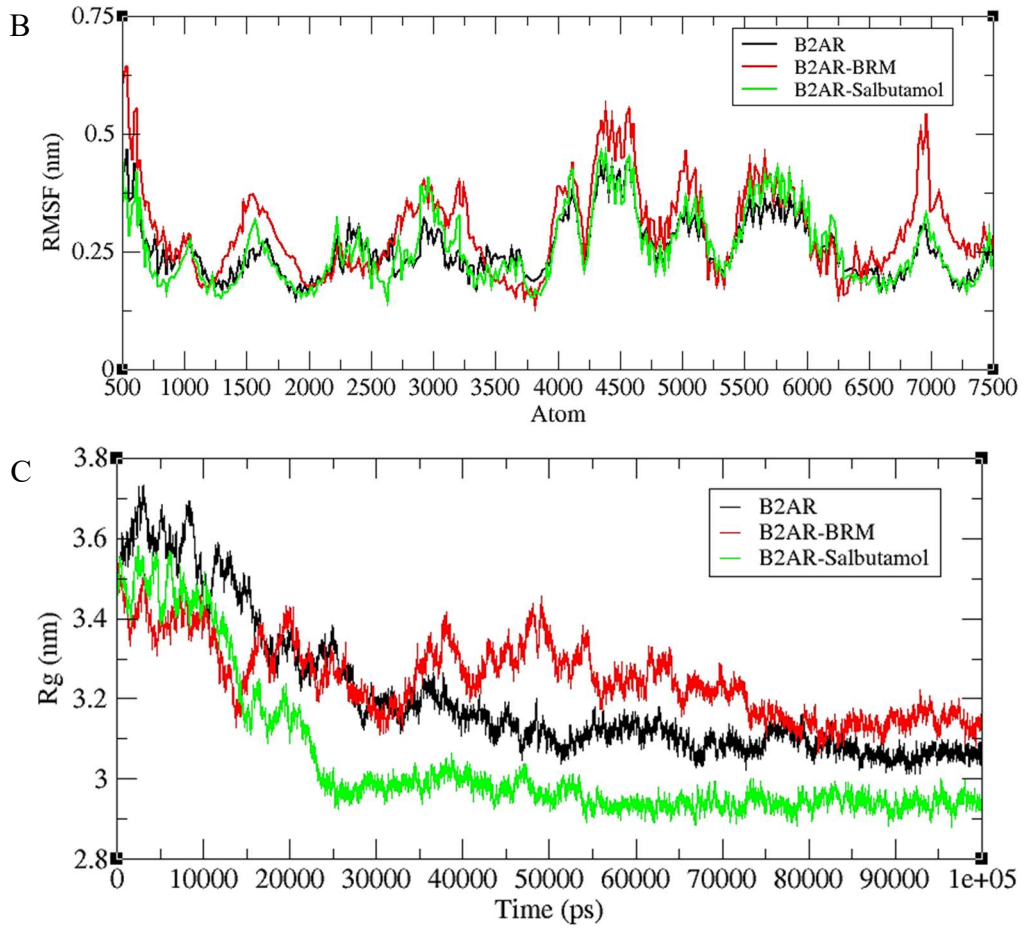


Figure 4. Trajectory analysis of B2AR, B2AR-BRM, and B2AR-SBM (A) RMSD, (B) RMSF, and (C) Radius of Gyration (Rg) on 100 ns dynamics simulations.

The Root Mean Square Deviation (RMSD) in molecular dynamics simulations shows the average deviation over the simulation time (100 ns) with the backbone of the protein residues as the reference. RMSD analysis between the protein and ligand-protein complexes was performed to determine the presence or absence of deviations and the stability and similarity of the structures during the simulation. Figure 4-A shows the trajectories of the three different systems. B2AR in the black graph shows the RMSD trend of the single protein or when it does not bind to the ligand. The B2AR protein stabilizes at an RMSD of approximately 40 ns at 1 nm. The red graph shows the deviation trend in the B2AR and BRM complexes; however, the graph appears to be less stable, with a fluctuation of approximately 0.25 ns. The interaction with the salbutamol standard increased the RMSD up to 1.5 nm but tended to be more stable than that with BRM.

The Root mean square fluctuations (RMSF) determine the deviation of the atomic position from the original point; the higher the fluctuation, the more flexible the atom. A similar pattern can be observed for the B2AR and B2AR-Salbutamol complexes in Figure 4-B. Meanwhile, BRM gives a more unstable residue interaction. In the atomic number range of 1250-2000 at TM2-TM3, it occurs with fluctuations increasing by about 0.15 nm in B2AR-BRM compared to B2AR. A high fluctuation occurs in the GPCR activation region corresponding to TM5-TM6 in the atomic number around 4000-5000. Furthermore, the highest fluctuation increase was observed at TM7 around atomic number 7000.

ADMET prediction

ADMET prediction was conducted for BRM and SBM using the DeepPK machine learning platform (https://biosig.lab.uq.edu.au/deeppk). The results are summarized in Table 2. Both compounds showed similar enteric absorption profiles, with predicted human intestinal absorption probabilities of 0.981 and 0.966 for BRM and SBM, respectively, and oral bioavailability above 50%. Regarding distribution, BRM demonstrated high blood–brain barrier (BBB) penetrability (Pr = 0.999), whereas SBM was predicted to be non-

penetrable (Pr = 0.003). BRM was identified as a non-substrate for P-glycoprotein (P-gp) (Pr = 0.252), in contrast to SBM, which is a P-gp substrate (Pr = 0.585). BRM showed a higher steady-state volume of distribution (5.14) and plasma protein binding (24.55) compared to SBM (2.78 and −6.89, respectively). Metabolically, BRM was predicted to act as a CYP2D6 inhibitor and substrate, while SBM showed no CYP2D6 or CYP3A4 involvement. Neither compound showed AMES mutagenicity at the predicted doses. Both compounds were predicted to carry respiratory toxicity risk (BRM: Pr = 0.975; SBM: Pr = 0.999).

Table 2. ADMET Prediction data of salbutamol and bromhexine.

Compounds		Salbutamol	Bromhexine
Absorption	Human intestinal absorption/ Pr	Absorbed/0.966	Absorbed/0.981
	Human oral bioavailability 50%/Pr	Bioavailable/0.529	Bioavailable/0.717
	P-glycoprotein substrate/Pr	Substrate/0.585	Non-substrate/0.252
Distribution	Blood-brain barrier/Pr	Non-penetrable/0.003	Penetrable/0.999
	Plasma protein binding	-6.89	24.55
	Steady state volume of distribution	2.78	5.14
Metabolism	CYP 2D6 inhibitor	Non-inhibitor	Inhibitor
	CYP 2D6 substrate	Non-substrate	Substrate
	CYP 3A4 inhibitor	Non-inhibitor	Non-inhibitor
	CYP 3A4 substrate	Non-substrate	Non-substrate
Excretion	Clearance	7.09	10.8
	Cation transporter 2/Pr	Non-inhibitor/0.099	Inhibitor/0.671
	Half-life of drug	< 3hs (Low confidence)	< 3hs (High confidence)
Toxicity	AMES mutagenesis/Pr	Safe/0.136	Safe/0.002
	Max. tolerated dose	-0.27	-0.07
	Respiratory disease/Pr	Toxic/0.999	Toxic/0.975

*Note: Pr = Probability

▪ DISCUSSION

The experiment demonstrated that ENDS was able to deliver aerosolized BRM by detecting it using a mass spectrometer. The pharmacokinetic data for BRM obtained during the COVID-19 pandemic revealed that the oral administration of 8 mg of BRM showed the blood concentration of 22.5 ± 7.50 ng/mL [28], while the cough syrup normally contains about 4 mg of BRM in each 5 mL [27]. In the present ENDS-based experiment, five puffs produced only 3.3156 ng/mL, which falls below therapeutic exposure. This discrepancy represents a key challenge in achieving pharmacologically relevant dosing through ENDS. Previous studies on nicotine have shown that aerosol deposition and systemic absorption are sensitive to flux and puff topography. A study by Bono et al. (2025) demonstrated that low-flux ENDS required more puffs and functioned as substitutes for cigarettes, while high-flux ENDS delivered greater suppression of withdrawal but were less tolerable because of irritancy. These findings suggest that maximizing ENDS as a therapeutic delivery platform will require balancing device parameters and patient usability to achieve both efficacy and adherence [34]. In vivo confirmation of ENDS-based API delivery beyond the nicotine models already established in the literature [35], remains necessary to validate this platform for therapeutic agents such as BRM.

These findings raise the question of whether the inhaled route could address the pharmacokinetic limitations of oral BRM. A meta-analysis of 24 RCTs involving 2,192 patients with COPD exacerbations

demonstrated with moderate certainty that mucolytics as a class improved treatment success rates and overall symptom scores; however, no significant effect on breathlessness was identified, and the analysis was limited by methodological heterogeneity across included studies [36]. Notably, this class-level evidence encompasses multiple agents including NAC, carbocysteine, and erdosteine, and the individual evidence base for bromhexine specifically remains comparatively limited. This evidence gap strengthens the rationale for exploring the inhaled route: pharmacokinetic data confirm that bronchial tissue concentrations reach 1.5–3.2 times the plasma concentration two hours after oral dosing [27], suggesting that inhalation-based delivery may theoretically achieve more targeted pulmonary concentrations with lower systemic exposure. Closing this gap will require dose-escalation studies correlating puff number and e-liquid concentration with delivered BRM.

In GPCRs such as β2AR, the transmembrane regions play a crucial role in regulating conformational changes that triggers receptor activation. Fluctuations in TM5 and TM6 are particularly significant because outward movement of TM6 and rearrangement of TM5 are essential steps in creating the binding pocket of the G protein and thereby stabilizing the active site of the receptor. Increased fluctuations in these helices, as observed in BRM, indicate flexibility that suggests weaker stabilization of the active conformation. In line with partial agonist behavior, decreased signaling effectiveness might result from lower stability of the active conformation [30],[37].

Because both complexes are unstable with the BRM, the residual RMSF value corresponds to the observed RMSD. The RMSF analysis shows that the interaction with BRM increases flexibility in several protein domains that allow BRM to act as a partial agonist or as an allosteric modulator. This result is supported by the Radius of Gyration (Rg) graph in Figure 4-C, which shows the atomic distance from the central atom with a value of approximately 2.9 nm occurring from 25 to 100 ns, where salbutamol consistently maintained a lower Rg than BRM, indicating greater protein compactness and stability. The interaction of β2AR with BRM increases Rg, indicating system instability. RMSD, RMSF, and Rg values support the partial-agonist or allosteric-modulator interpretation for BRM, based on greater fluctuation relative to the more stable salbutamol–β2AR complex. Other GPCR–ligand studies report a similar pattern: partial agonists show more backbone flexibility than full agonists. Those studies also calculate binding free energy (e.g., MM-PBSA) and run functional assays such as radioligand binding or cAMP accumulation to confirm the interpretation. This study did not include those analyses. Future functional studies should test the partial-agonist or allosteric-modulator hypothesis before treating it as a confirmed mechanism.

Several methodological constraints beyond the computational limitations should be acknowledged. The reported ENDS efficiency (99.8%) measures how much BRM left the e-liquid reservoir, not how much reached the trapped aerosol matrix. These are two different measures of delivery performance and should not be conflated. Particle size distribution of the aerosolized BRM was not measured in this study; confirming the fraction able to reach the lower respiratory tract would require MMAD and GSD characterization by next-generation impactor. Aerosol collection and quantification followed a single five-puff session without replicate sampling, precluding assessment of measurement variability.

The ADMET predictions add context for BRM as an inhaled therapeutic. BRM is not a P-glycoprotein substrate, unlike salbutamol, and has a higher predicted volume of distribution. Broader tissue penetration may support sustained mucolytic activity beyond the central airways. Two safety signals warrant attention in future studies: BRM's predicted high BBB penetrability (Pr = 0.999) and its CYP2D6 inhibitory profile, which may give rise to drug–drug interactions in COPD patients on multiple medications. The predicted respiratory toxicity for both compounds likely reflects high pharmacological activity in the airways rather than direct tissue damage, an interpretation supported by SBM's established clinical safety record as an approved inhaled bronchodilator. While the ADMET data do not preclude BRM as an inhaled mucolytic candidate, these findings underscore the necessity of structured inhalation toxicology and pharmacokinetic studies prior to clinical application.

CONCLUSION

This study revealed that ENDS could deliver BRM as an active pharmaceutical ingredient in the form of e-liquid. The HPLC-MS concentration assay detected that there is BRM post-delivery after 5 puffs. The ENDS devices might deliver API by aerosolizing the drug to the respiratory tract to achieve targeted local delivery. This targeted approach using ENDS is non-invasive and user-friendly, enhancing patient compliance. The possibility of a targeted therapeutic effect is also confirmed by the interaction of BRM as an API with B2AR on its active agonist site. As a result, the binding value is comparable to SBM as a standard drug, showing several hydrogen bond interactions. However, from the dynamic simulation, the BRM interaction with the protein does not form a stable complex, but it may act as an allosteric modulator or partial agonist. The lack of stable dynamic simulation does not diminish the clinical potential of BRM delivered by the device. Despite the controversy surrounding this device, its potential application as a drug delivery device should not be overlooked. These findings indicate that ENDS can deliver a non-nicotine mucolytic agent at a quantifiable, though currently sub-therapeutic, concentration, positioning this platform as a candidate delivery route for BRM pending further dose optimization. Closing the gap with oral therapeutic exposure will require dose-escalation experiments correlating puff number and e-liquid concentration with delivered BRM, alongside functional confirmation of receptor engagement given the unresolved mechanistic profile observed in the molecular dynamics analysis.

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