

Development of a Microemulsion High Performance Liquid Chromatography (MELC) Method for Determination of Salbutamol in Metered-Dose Inhalers (MDIS)

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ARTICLE INFO

Article Type:

Methodology

Article History:

Received: 25 Jan. 2013

Revised: 13 Feb. 2013

Accepted: 15 Feb. 2013

ePublished: 16 Feb. 2013

Keywords:

Microemulsion

HPLC

MELC

Determination

Validation

Salbutamol

ABSTRACT

Introduction: A sensitive and rapid oil-in-water (O/W) microemulsion high performance liquid chromatography (MELC) method has been developed. The water-in-oil (w/o) microemulsion was used as a mobile phase in the determination of salbutamol in aqueous solutions. In addition, the influence of operating parameters on the separation performance was examined. **Methods:** The samples were injected into C18, (250mm×4.6mm) analytical columns maintained at 25°C with a flow rate 1 ml/min. The mobile phase was 95.5% v/v aqueous orthophosphate buffer 20 mM (adjusted to pH 3 with orthophosphoric acid), 0.5% ethyl acetate, 1.5% Brij35, and 2.5% 1-butanol, all w/w. The salbutamol and internal standard peaks were detected by fluorescence detection at the excitation and emission wavelengths of 267 and 313 nm respectively. **Results:** The method had an accuracy of > 97.78% and the calibration curve was linear ($r^2 = 0.99$) over salbutamol concentrations ranging from 25 to 500 ng/mL. The intra-day and inter-day precisions (CV %) were <1.6 and <1.8, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were 9.61ng/ml and 29.13ng/ml, respectively. **Conclusion:** The method reported is simple, precise and accurate, and has the capacity to be used for determination of salbutamol in the pharmaceutical preparation.

Introduction

B2-adrenoreceptors are located in many tissues including the airway tissues. Salbutamol is a short acting β_2 -adrenoreceptor agonist. It stimulates β_2 -receptors located in the cell membrane of smooth muscles present in the airways resulting in bronchodilation, thereby providing relief for sufferers of asthma and other conditions associated with airway obstruction.

Salbutamol has a relative molecular mass of 239.31 (Fig. 1) and is a hydrophilic compound, which is relatively polar with basic properties. It has two ionizable groups: a secondary amine (pKa 10.3) and phenol (pKa 9.4). Salbutamol can be delivered to the site of action in a variety of ways such as oral and aerosol-inhaler systems. Inhaled salbutamol is the most commonly used quick-relief medication. Many HPLC methods were reported for the determination of salbutamol in aqueous and biological fluids. However, all the reported methods were performed using conventional mobile phase and HPLC methods.¹⁻⁸ The solvents that are usually used as mobile phase for the conventional HPLC, such as

acetonitrile, have properties that, from a 'green' analytical chemistry perspective, make them less preferable: they are flammable, volatile and toxic. Consequently, HPLC solvents including acetonitrile, THF and methanol must be disposed of as chemical waste which is costly and increases the laboratory's environmental waste-disposal burden. In addition, acetonitrile is an expensive solvent, as brought to the fore during a worldwide shortage in 2009, when limited availability caused prices to increase considerably.⁹ In contrast, microemulsion systems are environmentally friendly alternatives to the traditional solvents of the mobile phase for liquid chromatography. Recently, microemulsions have increasingly been used in pharmaceutical analyses. As demonstrated by isocratic O/W, microemulsions are used for the separation of mixtures of test solutes and pharmaceutical compounds.¹⁰⁻¹³ In this paper, it is proposed to extend the MELC method that has previously been published by authors¹⁴ to the analysis of salbutamol in aqueous solution and to study the effects of experimental parameters on the assay of salbutamol.

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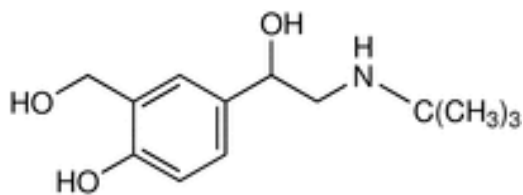


Fig. 1. Molecular structure of Salbutamol.^{15,16}

Experimental

Materials and chemicals

Salbutamol and bamethane sulphate were purchased from Sigma–Aldrich (Louis, USA). Ethyl acetate (Fisher Chemical), Brij35, and 1-butanol (HPLC grade) were supplied by Sigma–Aldrich (Louis, USA). All solutions were prepared with ultra-pure Milli-Q water obtained from a Milli-Q Water Millipore Purification System (USA).

Chromatographic conditions

HPLC system consisted of a Hewlett-Packard (HP) 1050 pump and an autosampler connected to an on-line membrane degasser (Thermo Separation Products, California, USA). The fluorescence detector (Shimadzu RF-551 Tokyo, Japan) had an excitation wavelength of 267nm and an emission wavelength of 313nm, and the detector was linked to Prime Multi-channel Data Station Software Version 4.2.0 (HPLC Technology Ltd, Herts, UK).

The mobile phase of 1.5:0.5:2.5:95.5 Brij35:ethyl acetate:butanole :phosphate buffer 20mM (adjust pH with orthophosphoric acid) (% w/w) was delivered at a constant flow rate of 1ml/min. The mobile phase was filtered under vacuum through a 0.45 μ m filter (Gelman Science, Germany) and degassed in an ultrasonic bath under vacuum for 10min. The samples were injected onto a 5 μ m Spherisorb C18 (250mm X 4.6mm) column at 25°C and the injection volume was 100 μ L. The salbutamol peak was detected by fluorescence detector at the excitation and emission wavelengths of 267 and 313 nm, respectively.

Preparation of standard salbutamol sulphate in mobile phase

A stock solution containing 100 μ g/mL salbutamol was prepared using the bamethane (as an internal standard) solution. The internal standard solution was prepared beforehand at concentration of 400 μ g/L in the mobile phase. Ten milliliters of stock solution was pipetted into a 100ml volumetric flask and made up to volume using the internal standard solution to produce a salbutamol of 10 μ g/ml (sub-stock). The calibration standards in the concentration range of 25 to 500ng/ml were prepared

using internal standard solution. All standards/samples were filtered through a 0.45 μ m filter prior injection.

Method development

The MELC method that we have previously published¹⁴ was further developed for the analysis of salbutamol in the pharmaceutical preparation. The effect of operating parameters on the speed and efficiency of chromatographic separation was studied.

Concentration of surfactant

Following the investigation of different concentrations of Brij35 (0.5% to 2% w/w), it was observed that the retention time for bamethane (internal standard) decreased upon increasing the concentration of Brij35 from 0.5% to 1%. This suggests that Brij35 may have modified the stationary phase surface¹⁴ and therefore reduced the retention time of bamethane. However further increases in Brij35 concentration had a very small effect on the retention time of both bamethane and salbutamol (Fig. 2).

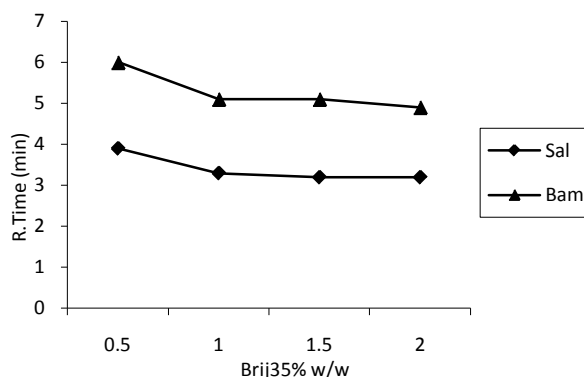


Fig. 2. Effect of Birj concentration.

Concentration of co-surfactant

Different concentrations of co-surfactant were studied. Fig. 3 shows the effect of changing the concentration of co-surfactant butanol across the range 0.5-3.5% w/w. It was found that the retention time of both salbutamol and bamethane decreased upon increasing the concentration of butanol from 0.5-2.5% w/w, which is due to an increase in the solubilization capacity of the microemulsion with the use of a co-surfactant.¹⁴ However, this concentration appeared to reach a maximum level and a further increase in butanol concentration showed no marked effect on the retention time (Fig. 3).

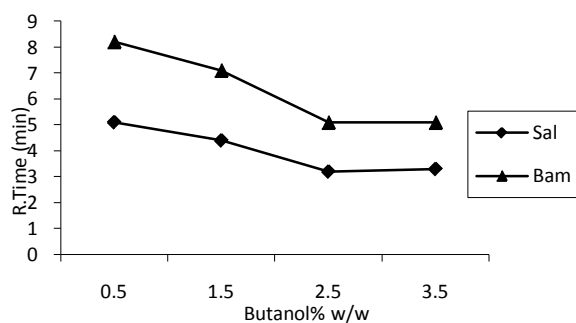


Fig. 3. Effect of cosurfactant concentration.

Oil concentration

Different concentrations of oil (ethyl acetate) were also investigated in the range 0.5-1.0% w/w (Fig. 4) and a slight decrease in retention of analytes was observed on increasing the oil content above 0.5% w/w. In contrast to lipophilic compounds, hydrophilic compounds such as salbutamol and bamethane appear to have a high affinity to the continuous phase of the microemulsion and therefore do not partition as fully they partition as in the oil droplet.^{14,17}

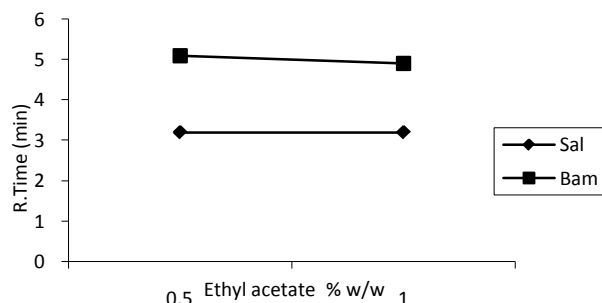


Fig. 4. Effect of oil concentration.

Buffer concentration

The effect of phosphate buffer concentration on retention behavior of both salbutamol and bamethane was studied at different concentration levels. Four mobile phases were prepared with different concentrations of phosphate buffer 5, 10, 20 and 25mM. Fig. 5 shows that retention time of both salbutamol and the internal standard decreased, as the buffer concentration increased. This is due to the fact that in reverse phase chromatography, the retention time of positively charged analytes decreases with the increasing buffer concentration.¹⁴

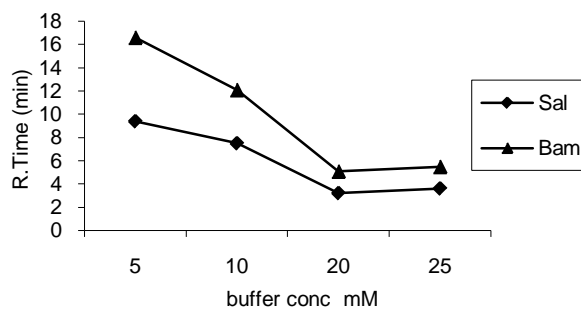


Fig. 5. Effect of buffer concentration.

Column temperature

The effect of temperature was examined at 6 different temperatures from 25-50°C (Fig. 6). It was found that increasing the temperature had no marked effect on the retention of both salbutamol and the internal standard. Peak efficiency and resolution were improved with increasing temperature. This result is consistent with findings reported by Althanyan *et al.*¹⁴

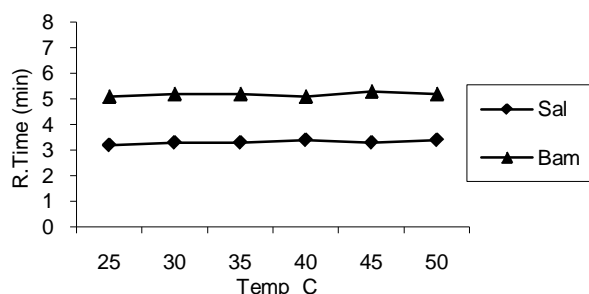


Fig. 6. Effect of temperature.

Method validation

The developed method was validated according to ICH¹⁸ guidelines.

Selectivity

The method was shown to be selective for salbutamol. Fig. 7 shows a typical separation of salbutamol (200ng/ml) and the internal standard bamethane (400ng/ml), all dissolved in the mobile phase. The figure shows that salbutamol was eluted at 3.2 min. The analysis of mobile phase and blanks confirmed that there were no interfering peaks due to the blank.

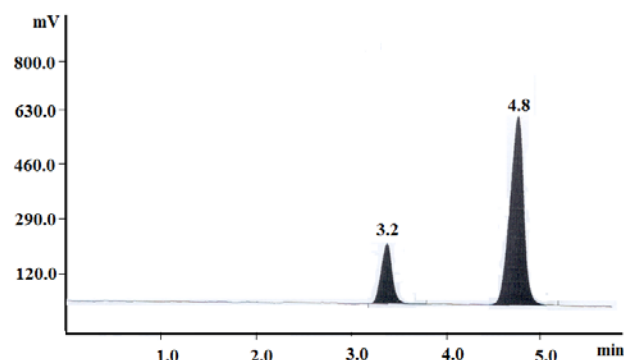


Fig. 7. Chromatogram of salbutamol (200 μ g/L), and the internal standard, bamethane (400 μ g/L). Peak identities: salbutamol 3.2min, and bamethane 4.8min.

Linearity

Six different concentrations were prepared ranging from 25 to 500ng/ml including the limit of quantification (LOQ) and covering the expected range. The linearity of the calibration standards was evaluated over this range. The calibration samples were injected in duplicates and blank samples were also analyzed along with the calibration standards. The detector response was shown to be linear over the range of 25 to 500ng/ml and gave a regression coefficient (r^2) of 0.9995 and $y = 0.0013x + 0.0467$.

Sensitivity

The measurements of limit of detection (LOD) and limit of quantification (LOQ) were based on the standard deviation (SD) of the y intercept from the regression of the calibration curve.¹⁹ The limit of detection equal to 3.3s/m and limit of quantification equal to 10s/m were given where, s is the standard deviation of y-intercept and m is the slope of the calibration. The limit of detection (LOD) was 9.61ng/ml and the limit of quantification was 29.13ng/ml.

Precision

Precision was assessed by five determinations at known concentrations corresponding to low (25ng/ml), medium (250ng/ml) and high (500ng/ml) levels in the calibration range. The same study was repeated for 5 days to determine the inter-day variation. The intra- and inter-day variations were determined by calculating the relative standard deviation. The intra-day (RSD %) and inter-day (RSD %) variations are shown in Table 1. The low values of RSD indicate the precision of method.

Accuracy

The accuracy of method was determined by adding the analyte into blank matrices at different concentrations;

then comparing the measured spiked concentration with the true concentration of salbutamol. Three different concentration levels corresponding to low (25ng/ml), medium (250ng/ml) and high (500ng/ml) were used (n=5 for each level). The accuracy of the method ranged from 97.78 to 99.74% (Table 2).

Table 1. Intra-day and inter-day precision of the MELC method

Nominal concentration (ng/ml)	Intra-day coefficient of variation (%)	Inter-day coefficient of variation (%)
25	0.2	1.8
250	1.6	1.2
500	0.9	0.7

Table 2. Accuracy of the MELC method

Actual concentration (ng/ml)	Observed concentration (ng/ml)	Accuracy %
25	29.33	97.78
250	119.69	99.74
500	246.29	98.51

Stability

Reference solutions were stored in the refrigerator at +4°C for 6 weeks and re-analyzed in an injection sequence employing freshly prepared standard solutions. The concentration after such storage conditions and on comparison with a freshly prepared standard was 99% w/w. Longer storage periods may be possible but were not assessed in this study.

Robustness

The robustness of an analytical method is a measure of its capacity to resist changes due to small variations in method conditions. The method robustness was assessed as a function of changing the Brij35, buffer concentration, butanol and pH. The changes ranged over $\pm 5\%$ of the target (optimized chromatographic experimental condition). The method system suitability criteria of a resolution greater than 2.0 between the peaks were maintained throughout these experiments.

Table 3. Aerodynamic characteristics of the emitted dose of salbutamol from pMDI with an attached Aerochamber Plus at flow rate of 28.3L/m

Run No.	Amount left in spacer (% of the label dose)	Delivered amount (% of the label dose)	Fine particle dose (μg)	Fine particle fraction (% of the label dose)	MMAD (μm)	GSD
Run 1	34%	69%	57	82	2.6	1.7
Run 2	27%	76%	67	88	2.8	1.5
Run 3	30%	80%	68	85	2.9	1.5
Mean	30%	75%	64	85	2.77	1.57

Application of method to a salbutamol pharmaceutical product

Andersen MKII Cascade Impactor (ACI) was used to assess the in-vitro dosing characteristics of the emitted dose from a salbutamol pMDI (Ventolin Evohaler™; GlaxoSmithKline, Brentford, UK) with an attached AeroChamber Plus ® (valve holding chamber). The aerodynamic characteristics include the measurements of mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD) and geometric standard deviation (GSD). This proposed MELC method was used to assay the amount of salbutamol that was deposited on different stages of the ACI. The PMDI salbutamol inhaler was attached with the AeroChamber Plus ® spacer device, having initially been shaken for five seconds, to a mouth piece adaptor. The presence of the mouth piece adapter ensures that there is no sample loss between the collection tube and the inhaler mouth piece. The flow rate through the mouth piece was set at 28.3L/min. The inhaler was actuated and the test flow duration was adjusted for 8.5s to achieve an inhaled volume of 4L drawn through the inhaler. The next dose was actuated after eight seconds elapse, and a total of five doses were actuated. Each actuation delivered a salbutamol sulphate equivalent to 100 μg salbutamol per dose; then the inhaler was removed and the pump switched off.

The mean (n=3) fine particle dose (the amount of salbutamol that has aerodynamic particle diameters less than 5 microns), mass median aerodynamic diameters (the diameter at which 50% of the salbutamol particles by mass are larger and 50% are smaller), and the geometric standard deviation (a measure of the spread of an aerodynamic particle size distribution) for salbutamol were 64, 2.77 and 1.57 μg , respectively (Table 3).

Conclusion

This study has shown that our previously published method was successfully extended for the determination

of salbutamol in aqueous solutions and a pharmaceutical preparation. The method was robust for the experimental operating conditions over a range of $\pm 5\%$ of the target (experimental condition). The method was rapid, precise and accurate. It has been shown that this method could be used for quality control for the determination of the content of the emitted dose and dose content uniformity in the inhalation devices delivering salbutamol.

Competing interests

Authors declare they have no competing interests.

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