Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 397–402

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Microemulsion high performance liquid chromatography (MELC) method for the determination of terbutaline in pharmaceutical preparation

M.S. Althanyan[a], K.H. Assi[a,*], B.J. Clark[a], J. Hanaee[b]

[a] Bradford School of Pharmacy, School of Life Sciences, Bradford BD7 1DP, UK
[b] School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Article history:
Received 14 June 2010
Received in revised form 19 January 2011
Accepted 22 January 2011
Available online 28 January 2011

Keywords:
Microemulsion
HPLC
MELC
Determination
Validation
Terbutaline

ABSTRACT

A robust and sensitive microemulsion HPLC (MELC) method using oil-in-water microemulsion mobile phase was developed and used for the determination of terbutaline in Bricanyl® Turbuhaler. The applicability of microemulsion as an eluent for reversed phase HPLC was examined. In addition, the effect of operating parameters on the separation behaviour was studied.

The samples were injected into C18 Spherisorb (250 mm × 4.6 mm × 5 μm) columns at 25 °C using a flow rate of 1 ml/min. The mobile phase was 95.5% aqueous orthophosphate buffer (adjusted to pH 3 with orthophosphoric acid), 0.5% ethyl acetate, 1.5% Brij35, and 2.5% 1-butanol, all w/w. The terbutaline peak was detected by fluorescence, using excitation and emission wavelengths of 267 and 313 nm, respectively.

The accuracy of method was >99% and the calibration curve was linear (r^2 = 0.99). The limit of detection (LOD) and limit of quantitation (LOQ) were 8 μg/L and 26 μg/L, respectively. The intra-day and inter-day precisions (in term of % coefficient of variation) were < 1.46% and <0.97%, respectively. The influence of the composition of the microemulsion system was also studied and the method was found to be robust with respect to some changes of the microemulsion components. The microemulsion HPLC method has been applied to determine the content of the emitted dose and the fine particle dose of terbutaline in a Bricanyl® Turbuhaler.

1. Introduction

Microemulsion is a transparent and thermodynamically stable system. It contains submicron droplets that are dispersed in an immiscible liquid. Oil-in-water microemulsions are composed of submicrometer oil droplets that are dispersed throughout an aqueous continuous phase. The droplets are covered by a shell consisting of a suitable surfactant and a co-surfactant. The surfactant molecules form interface film that separates the oil phase from the aqueous continues phase. This film has a low surface tension in the oil–water mixture. The addition of co-surfactant reduces the interfacial tension further as it locates itself at the oil-water interface and therefore lowers the interfacial free energy which favours the formation of stable microemulsion [1]. In reversed phase HPLC, the stationary phase is non-polar, while the mobile phase is relatively polar. Hence the high aqueous content in O/W microemulsion has made this mobile phase very compatible with the reversed phase HPLC [2]. Microemulsions have received much interest in different fields of science. Microemulsions have been used for many applications: in drug delivery and to enhance drug solubilisation [3], in cosmetics as personal care formulations, and for a number of other applications [4–6]. In recent years, microemulsion liquid chromatography (MELC) has been increasingly used in pharmaceutical analysis. O/W microemulsion was used as a mobile phase for the separation of mixtures of test solutes or pharmaceutical compounds by isocratic HPLC system [7–10], and for the determination of drugs in their pharmaceutical preparations [11–13]. Several other studies have used gradient MELC for the separation of different ranges of pharmaceutical compounds [14,15] and for quantification of drugs in their pharmaceutical preparations [2]. Although gradient MELC has been reported to have superb power to separate analytes with different polarity, McEvoy et al. [16] have found that the peak retention times and resolution were irreproducible. The authors attributed this to the nature of the absorbed layer on the column packing and to the possibility that gradient elution can cause a breakdown of microemulsion system. They also stated that reproducibility can be achieved by allowing the column to equilibrate with the microemulsion mobile phase and a constant adsorbed layer on the packing. Previous studies using microemulsions as the mobile phase for HPLC have used SDS as a surfactant but we found (unpublished data) that this mobile phase was not able to separate a highly hydrophilic compounds that have very similar chemical properties. Marsh et al. [15] reported a similar observation. Terbutaline sulphate is a selective β_2-adrenoceptor

* Corresponding author.
E-mail address: khaassi@Bradford.ac.uk (K.H. Assi).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.01.027

agonist that is used as a bronchodilator. Terbutaline is available as the Bricanyl® Turbuhaler®, a multi-dose reservoir inhaler device releasing 500 μg of micronised terbutaline sulphate per inhalation.

In this work, non-ionic surfactant was used in the formation of microemulsion and the potential of using microemulsion as an eluent for HPLC for the determination of terbutaline in the Bricanyl® Turbuhaler was examined. Moreover, the effect of operating parameters on the separation performance was studied.

2. Experimental

2.1. Materials and chemicals

Terbutaline hemisulphate salt 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).

2.2. Chromatographic conditions

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

Chromatographic separation was performed using a 250 mm × 4.6 mm i.d. (5 μm particle size) Spherisorb C18 column (Waters, UK). The mobile phase was prepared by weighting 1.5%w/w of Brij35, 2.5%w/w of 1-butanol, 0.5%w/w of ethyl acetate, which then dissolved in 95.5%w/w of 20 mM orthophosphate buffer (adjusted to pH 3 with orthophosphoric acid). The solution was then sonicated for 15 min. The mobile phase was filtered under vacuum through a 0.45 μm filter (Gelman Science, Germany) and degassed in an ultrasonic bath under vacuum for 10 min. Terbutaline samples and bamethane (as an internal standard) were injected into the system and separated at 25 °C. The mobile phase was delivered at a flow rate of 1.0 mL/min and the injection volume was 20 μL.

2.3. Particle size measurement of the mobile phase

The mobile phase (see Section 2.2) was filtered through 0.2 μm filters. The reported size was the Z-average size (cumulants mean) of five replicates determined at 25 °C based on PCS using a Zetasizer Nano ZS® (Malvern, UK).

2.4. Preparation of standard terbutaline sulphate in mobile phase

A stock solution containing 100 μg/mL of terbutaline was prepared using the internal standard solution. The internal standard solution was prepared beforehand at concentration of 400 μg/L in the mobile phase. Ten millilitres of stock solution were pipetted into a 100 mL volumetric flask and made up to volume using the internal standard solution to produce a terbutaline sulphate of 10 μg/mL (sub-stock). Calibration standards in the concentration range of 25, 50, 100, 200, 300, 400 and 500 ng/mL were prepared in the appropriate volumetric flasks using internal standard solution. All standards/samples were filtered through a 0.45 μm filter prior injection.

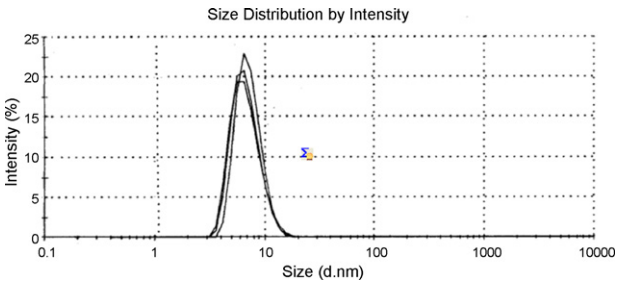

Fig. 1. Size distribution (by intensity) of nanoemulsion mobile phase. The mobile phase consists of 1.5:0.5:2.5: 95.5 Brij35: ethyl acetate: 1-butanol: water with (TFA to adjust pH) (% w/w) measured by Malvern Zetasizer Nano analysis.

3. Result and discussion

3.1. Particle size of the mobile phase

The preparation of the mobile phase was repeated on five different occasions, and five replicate measurements were performed for each mobile phase. The particle size obtained for all mobile phases was always less than 10 nm. Fig. 1 shows a representative measurement of particle size of the mobile phase. On the other hand, the mobile phase was stable throughout the study period.

3.2. Optimisation of mobile phase

3.2.1. Concentration of surfactant

The presence of surfactant in the mobile phase can affect the separation selectivity. The surfactant molecules have a tendency to adsorb on the surface of the porous stationary phase and modify their surfaces [7]. The adsorbed surfactant molecules fill up part of the silica pore volume; hence they reduce stationary phase surface area and increase the thickness of the stationary organic layer, and therefore change the efficiency of the ODS column [7,17]. The adsorbed surfactant on the stationary phase could have a direct impact on the retention of solutes and their partition with the stationary phase. Different concentrations of Brij35 were investigated (see Fig. 2). It was found that the retention of bamethane decreased with increasing the concentration of Brij35 from 0.5% to 1%. This shows that Brij35 may have modified the stationary surface and therefore reduced the retention time of bamethane. However, further increase of Brij35 concentration has a very small effect on the retention time of both bamethane and terbutaline.

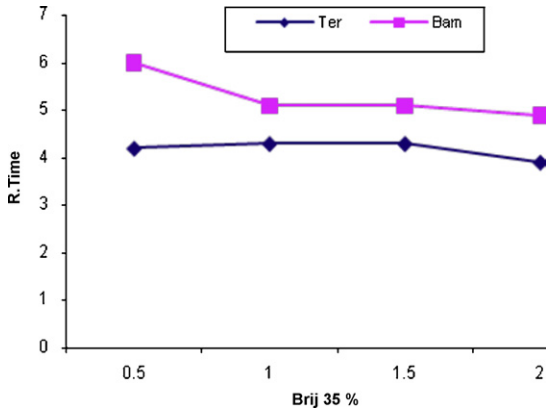

Fig. 2. Effect of Brij concentration; Ter: terbutaline, Bam: bamethane.

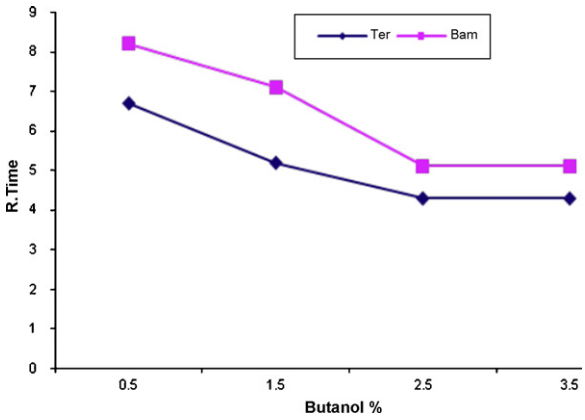

Fig. 3. Effect of cosurfactant concentration Ter: terbutaline, Bam: bamethane.

3.2.2. Concentration of the co-surfactant

A co-surfactant such as alcohol is used to enhance and stabilise the microemulsion system. The nature of the co-surfactant affects the phase behaviour in the microemulsion system [18]. Fig. 3 shows the effect of changing the concentration of co-surfactant butanol in the range of 0.5–3.5%w/w. It was found that retention time of both terbutaline and bamethane decreases with increasing the concentration of butanol between 0.5 and 2.5%w/w. Nevertheless, a further increase of butanol concentration has shown no marked effect on the retention time (Fig. 3). The decreases in retention time with increasing the concentration of co-surfactant could be attributed to the increase of solubilisation capacity of the microemulsion with the use of butanol.

3.2.3. Oil concentration

The oil is dispersed into nano-droplets in the continuous water phase to form a nanoemulsion through the assistance of the surfactant/co-surfactant which resides on the oil–water interface [19]. In microemulsion HPLC, the type and concentration of oil have a marked effect on the retention time of analytes. This effect depends on the nature of these analytes. Different concentrations of oil were studied in the range of 0–1%w/w (Fig. 4). When the concentration of oil is zero, the mobile phase will contain micelles. The micellar mobile phase gave longer retention times (5.2 and 7.3 min for both terbutaline and bamethane, respectively) compared to microemulsion mobile phase. The addition of oil decreases the retention of both analytes. This is due to the fact that microemulsion has a stronger elution capacity than that of the micellar solution

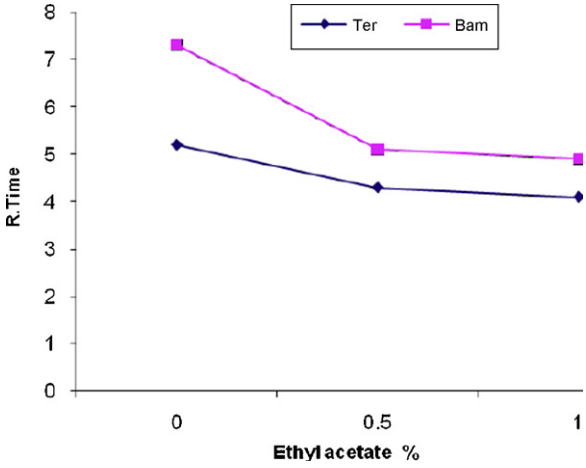

Fig. 4. Effect of oil concentration; Ter: terbutaline, Bam: bamethane.

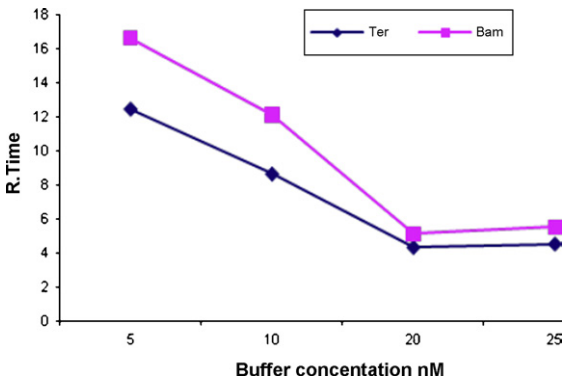

Fig. 5. Effect of buffer concentration; Ter: terbutaline, Bam: bamethane.

[20]. A slight decrease in retention of analytes was observed with increasing the oil content above 0.5%. Unlike lipophilic compounds, hydrophilic compounds such as terbutaline and bamethane have a high affinity for the continuous phase of the microemulsion and therefore they are not partitioned as fully in the oil droplet [21].

Other types of oil such as octane, heptanes and hexane were assessed but none of these oils was able to form microemulsion in the presence of Brij35.

3.2.4. Mobile phase pH

The effect of the pH of the mobile on the retention time of both terbutaline and bamethane was assessed at low pH (pH 3) and high pH (pH 6). It was found that there is no marked effect on the retention of terbutaline and bamethane with changing the pH. Both terbutaline and bamethane are weak basic drugs and they are fully protonated in the examined pH range. Hence, they will have less interaction with the ODS stationary phase and they have less affinity to the oil droplet. Therefore, changing the pH has a minimum effect on their retention. On the other hand, it was noticed that peak efficiency of terbutaline was improved at pH3. Lowering the pH of the mobile phase reduces the adsorption of the basic drugs to the silanol group of the stationary phase. Assi et al. [22] have used a low pH mobile phase for the determination of formoterol and budesonide in the Symbicort Turbuhaler to avoid the adsorption problem. The author indicated that the very low pH mobile phase eliminates the interaction between the ionised silanol group and the NH2 groups of the solutes.

3.2.5. Buffer concentration

The effect of phosphate buffer concentration on the retention behaviour of both terbutaline and bamethane was studied at different concentrations levels. Four mobile phases were prepared with different concentrations of phosphate buffer: 5, 10, 20 and 25 mM. The optimum buffer concentration was 20 mM. Fig. 5 shows that retention time of both terbutaline and the internal standard decreased as the buffer concentration increased. These results corroborate with the finding reported by Mao et al. [23]. However, Mao et al. have studied the effect of buffer concentration using conventional mobile phase. The consistency in both studies proves that, in reverse phase chromatography, the retention time of positively charged analytes decreases with increasing buffer concentrations whether the mobile phase contains microemulsions or not. This shows that there is an electrostatic interaction between protonated analytes (terbutaline and bamethane) and the silanol group even with the low pH mobile phase. The logarithm of the retention factor of a cationic analyte has a negative relationship with the logarithm buffer concentration in the cationic exchange column [23]. Mao et al. also reported that even with double end capping ODS there is still a considerable cation exchange between the positively charged

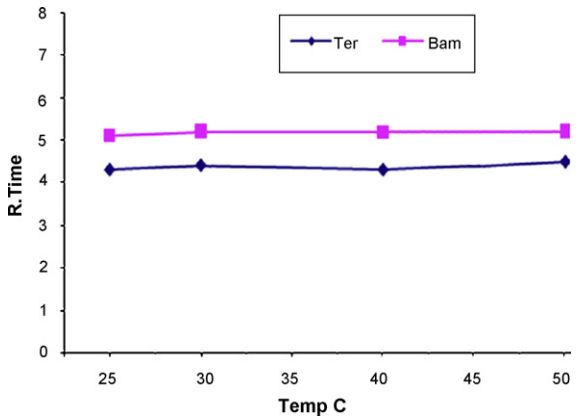

Fig. 6. Effect of temperature; Ter: terbutaline, Bam: bamethane.

analytes and the stationary phase, which the authors referred to as electrostatic interactions between the charged analytes and residual silanol groups.

3.2.6. Column temperature

The column temperature affects the elution of basic drugs in reverse phase chromatography. Changing the temperature of the column alters the dissociation constant of the basic analytes. The aqueous p*K*a of basic analytes decreases significantly with an increase in temperature, thus as temperature increases more of the neutral form and less of the protonated form will be present [24]. In the reversed phase, the main factor that controls the retention of analytes is their interaction with the stationary phase, and the neutral form interacts with the ODS phase much more strongly than does the charged form. Hence retention should increase upon increasing the temperature on the ODS column [23]. However, the effect of temperature on retention of basic drugs in an HPLC microemulsion system can be more complicated than that described above. In reversed phase microemulsion there are two contradictory mechanisms. On one hand, as the basic drugs become more neutral, they retain longer in the stationary phase. On the other hand, their partition with the oil droplet will increase and therefore their retention should decrease.

The effect of temperature was examined at four different temperatures: 20 °C, 30 °C, 40 °C and 50 °C (Fig. 6). It was found that increasing the temperature has no marked effect on the retention of either terbutaline or the internal standard. Peak efficiency and resolution were improved with increasing temperature. This result is consistent with findings reported by Marsh et al. [10]

3.3. Assay validation

The developed method was validated to determine the terbutaline in Bricanyl® Turbuhaler, and the validation procedure was based on ICH (1996) guidelines [25].

3.3.1. Selectivity

The method was shown to be selective for terbutaline. Fig. 7 shows a typical separation of terbutaline (200 μg/L) and the internal standard bamethane (400 μg/L), all dissolved in the mobile phase. The figure shows that terbutaline was eluted at 4.3 min. The analysis of mobile phase and blanks confirmed that there were no interfering peaks due to the blank.

3.3.2. Linearity

Six different concentrations were prepared to range from 25 to 500 μg/L including the limit of quantitation (LOQ) and covering the expected range. The linearity of the calibration standards

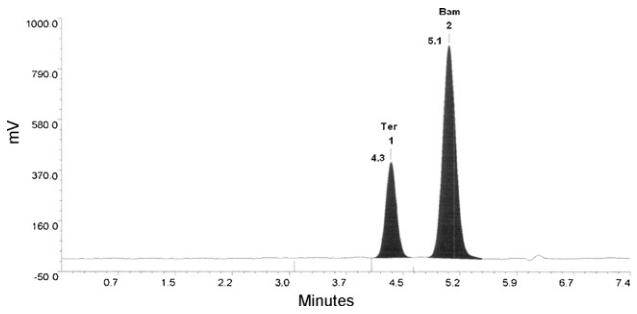

Fig. 7. Chromatogram of terbutaline (200 μg/L), and the internal standard, bamethane (400 μg/L). Peak identities: terbutaline 4.3 min, and bamethane 5.1 min.

was evaluated over this range. The calibration samples were injected in duplicates and also blank samples were analysed along with the calibration standards. The detector response was shown to be linear over the covered range and gave a regression coefficient (r^2) of 0.998. The standard deviations for the slope and intercepts were 0.00013 and 0.00814, respectively [$Y = 0.0032(\pm 0.00013)X - 0.002(\pm 0.00814)$]. $y = 0.0032x - 0.002$.

3.3.3. Sensitivity

The sensitivity was expressed as LOQ and limit of detection (LOD). LOQ is the injected amount that results in a peak with a height at least 10 times as high as the baseline noise level, and the LOD as peak height to base line ratio of 3:1 [25]. Another approach to calculate LOQ and LOD is based on the standard deviation (SD) of *y*-intercept from the regression of the calibration curve [26]. In this approach the LOQ = 10 s/m and LOD = 3.3 s/m where, *s* is the standard deviation of *y*-intercept and *m* is the slope of the calibration. The limit of detection (DL = 3.3 s/m) was 8 μg/L and the limit of quantitation (QL = 10 s/m) was 25 μg/L. Three samples of both terbutaline and bamethane were prepared at the quantitation limits and were analysed ($n = 10$), the relative standard deviation (R.S.D.) was 0.92%.

It was possible to use only one dose from Bricanyl® Turbuhaler in the measurement of the particle size distribution (see Section 4) due to the excellent sensitivity of the assay method. Otherwise more doses would have been required to be discharged into Andersen Cascade Impactor (ACI) which could overload the ACI stages and hence cause the particles to bounce off and re-entrain into the air stream. As a result, the particles will be carried to downstream stages which will introduce error in the size distribution measurement [27].

3.3.4. Precision

Precision was assessed by five determinations at known concentrations corresponding to low (25 μg/L), medium (200 μg/L) and high (500 μg/L) 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 variations (RSD %) ranged from 0.76 to 1.46% and inter-day RSD% ranged from 0.35 to 0.97% (Table 1).

Table 1
Intra- and inter-assay precision data for the NELC method.

Nominal concentration (ng/ml)	Intra-day coefficient of variation (%)	Inter-day coefficient of variation (%)
Low = 25	1.46	0.97
Medium = 200	1.22	0.80
High = 500	0.76	0.35

Table 2
Accuracy data for terbutaline.

Actual concentration (μg/L)	Observed concentration (μg/L)	% Accuracy
25	24.965	100.44
200	203.204	101.60
500	499.344	99.82

3.3.5. Accuracy

The accuracy of the method was performed by adding the analyte into blank matrices at different concentrations then it was assessed by comparing the calculated spike concentration with the true concentration of terbutaline. Three different concentrations levels corresponding to low (25 μg/L), medium (200 μg/L) and high (500 μg/L) were used ($n = 5$ for each level). The accuracy of the method ranged from 99.82 to 101.60% (Table 2).

3.3.6. Recovery

The recovery was assessed by extracting known amounts of terbutaline from membrane filters. The mean recoveries of terbutaline from the filters were > 98.04%. The details of recovery study for terbutaline from filters are shown in Table 3.

3.3.7. Stability

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

3.3.8. 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 pH, Brij35, 1-butanol and buffer concentration, the changes were over a range of ±5% of the target (experimental condition). The method system suitability criteria of a resolution greater than 2.0 between the peaks were maintained.

4. Application of the method

The pharmaceutical performance of inhaled products can be characterised by the total emitted dose and the aerodynamic particle size distribution including the fine particle dose. This MELC method was used to assay the content uniformity of the emitted dose and the fine particle dose of terbutaline in Bricanyl® Turbuhaler.

4.1. Dose content uniformity

The method was useful to measure the emitted dose of terbutaline in Bricanyl® Turbuhaler. The emitted dose uniformity was measured using a dose sampling apparatus described in pharmacopoeial methods (EP 2008, USP 2005) [28,29].Ten individual doses (dose number 2, 3, 4,49, 50, 51, 52, 98, 99 and 100) of the entire dose available (100 doses) were collected from the Bricanyl at a pressure drop of 4 kPa across the inhaler. The flow duration was 4.5 s; this was to allow a volume of 4 L to be drawn through the inhaler.

Each dose was collected and then was transferred to a 25 ml volumetric flask. It was diluted up to volume with internal standard solution (400 μg/L, bamethane), to give concentration of 500 μg/L.

The HPLC data was then compared with the label claim dose of Bricanyl inhaler (Table 4). The R.S.D. value is high because of the high inter-dose emission variability from a Turbuhaler inhaler [22,30].

Table 3
Recovery of terbutaline from membrane filters ($n = 5$).

Nominal concentration (μg/L)	Mean calculated concentration (μg/L)	% Recovery
100	101.53	101.53
200	196.15	98.08
500	490.19	98.04

Table 4
Percentage of the nominal dose of terbutaline emitted from Bricanyl Turbohaler at a pressure drop of 4 kPa across the inhaler.

Dose no.	% Nominal dose
2	65.2
3	47.3
4	63.6
49	92.2
50	76.7
51	64.7
52	67.0
98	73.5
99	84.5
100	82.1
Mean	71.7
SD	12.8
RSD	17.8

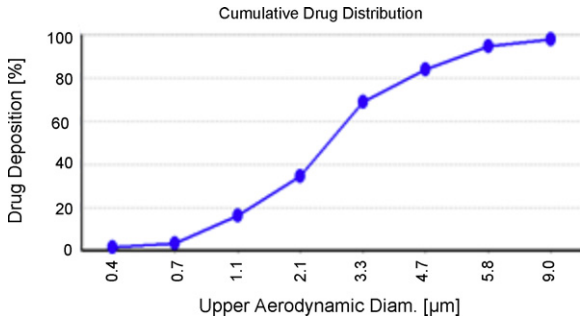

Fig. 8. Represents the cumulative drug distribution.

4.2. Particle size distribution

The particle size distribution and the fine particle mass from the Bricanyl® Turbuhaler were measured using the Andersen MKII Cascade Impactor. The Anderson cascade impactor was set up as described in the pharmacopoeia methods (EP 2008, USP 2005) [28,29]. The flow rate through the mouthpiece was set at a pressure drop of 4 kPa across the inhaler. Five separate determinations were made and for each determination one dose was discharged into the Andersen MKII Cascade Impactor. For each dose the pump was switched on for 4.5 s (equivalent to an inhaled volume of 4 L drawn through the inhaler) with the inhaler in situ ready to deliver each dose. The fine particle dose for terbutaline was 170.26 μg. The probability of the cumulative percentage of mass less than a stated particle size was plotted against the log of aerodynamic diameter (μm) as shown in Fig. 8. The mass median aerodynamic diameter (MMAD) was 2.76 and the geometric standard deviation (G.S.D.) was 1.79.

5. Conclusions

This study has shown that microemulsion can be used as a mobile phase for the analysis of drugs in their pharmaceutical preparation. Oil-in-water microemulsion was applied as a mobile phase and the method was successfully developed and validated.

The separation was highly robust to wide range of changes in temperature. MELC offered a fast analysis time for the determination of terbutaline in the aerosol formulation. Moreover, the method is reliable, precise, and accurate.

References

[1] L. Prince, Microemulsion Theory and Practices, Academic Press INC, New York, 1977, ISBN 0-12-565750-1.
[2] E. McEvoy, S. Donegan, J. Power, K. Altria, Optimisation and validation of a rapid and efficient microemulsion liquid chromatographic (MELC) method for the determination of paracetamol (acetaminophen) content in a suppository formulation, J. Pharm. Biomed. Anal. 44 (2007) 137–143.
[3] M.R. Gasco, Microemulsions in the Pharmaceutical Field: Perspectives and Applications, in: Industrial Applications of Microemulsions, Marcel Dekker Inc., New York, 1997, pp. 97–122.
[4] O. Sonneville-Aubrun, J.-T. Simonnet, F. L'Alloret, Nanoemulsions: a new vehicle for skincare products, Adv. Colloid Interface Sci. 108–109 (2004) 145–149.
[5] G. Lee, T. Tadros, Formation and stability of emulsions produced by dilution of emulsifiable concentrates. Part I. An investigation of the dispersion on dilution of emulsifiable concentrates containing cationic and non-ionic surfactants, Colloids Surf. 5 (1982) 105–115.
[6] X. Liu, Y. Guan, Z. Ma, H. Liu, Surface modification and characterization of magnetic polymer nanospheres prepared by miniemulsion polymerization, Langmuir 20 (2004) 10278–10282.
[7] A. Berthod, M. De Carvalho, Oil in water microemulsions as mobile phases in liquid chromatography, Anal. Chem. 64 (1992) 2267–2272.
[8] A. Berthod, J.J. Laserna, I. Carretero, Oil-in-water microemulsions as mobile phases for rapid screening of illegal drugs in sports, J. Liq. Chromatogr. 17 (1992) 3115–3127.
[9] K.D. Altria, A. Marsh, B.J. Clark, High performance liquid chromatographic analysis of pharmaceuticals using oil-in-water microemulsion eluent and monolithic column, Chromatographia 63 (2006) 309–314.
[10] A. Marsh, K.D. Altria, B.J. Clark, Oil-in-water microemulsion high performance liquid chromatographic analysis of pharmaceuticals, Chromatographia 59 (2004) 531–542.
[11] A. Malenovic, D. Ivanovic, M. Medenica, B. Jancic, S. Markovic, Retention modelling in liquid chromatographic separation of simvastatin and six impurities using a microemulsion as eluent, J. Sep. Sci. 27 (2004) 1087–1092.
[12] D.T. El-Sherbiny, M.I. Eid, D.R. El-Wasseef, R.M.M. Al-Ashan, F. Belal, Analysis of flunarizine in the presence of some of its degradation products using micellar liquid chromatography (MLC) or microemulsion liquid chromatography (MELC)—application to dosage forms, J. Sep. Sci. 28 (2005) 197–202.
[13] M.I. Walash, F. Belal, N. El-Enany, A. Abdelal, Microemulsion liquid chromatographic determination of nicardipine hydrochloride in pharmaceutical preparations and biological fluids. application to stability studies, J. Liq. Chromatogr. Rel. Technol. 30 (2007) 1015–1034.
[14] S.M. Bryant, K.D. Altria, An initial assessment of the use of gradient elution in microemulsion and micellar liquid chromatography, J. Sep. Sci. 27 (2004) 1498–1502.
[15] A. Marsh, B.J. Clark, K.D. Altria, Oil-in-water microemulsion LC determination of pharmaceuticals using gradient elution, Chromatographia 61 (2005) 539–547.
[16] E. McEvoy, S. Donegan, J. Power, K. Altria, Application of MELC and MEEKC for the analysis of paracetamol and related impurities in suppositories, Chromatographia 68 (2008) 49–56.
[17] A. Berthod, Causes and remediation of reduced efficiency in micellar liquid chromatography, J. Chromatogr. A 780 (1997) 191–206.
[18] N. Garti, A. Yaghmur, M.E. Leser, V. Clement, H.J. Watzke, Improved oil solubilization in oil/water food grade microemulsions in the presence of polyols and ethanol, J. Agric. Food Chem. 49 (2001) 2552–2562.
[19] T.G. Mason, J.N. Wilking, K. Meleson, C.B. Chang, S.M. Graves, Nanoemulsions: formation, structure, and physical properties, J. Phys.: Condens. Matter 18 (2006) R635–R666.
[20] J. Liu, J. Sun, Y. Wang, X. Liu, Y. Sun, H. Xu, Z. He, Characterization of microemulsion liquid chromatography systems by solvation parameter model and comparison with other physicochemical and biological processes, J. Chromatogr. A 1164 (2007) 129–138.
[21] R. Ryan, S. Donegan, J. Power, E. McEvoy, K. Altria, An Introduction to Microemulsion HPLC (MELC), LC-GC Europe, October 2008, pp. 502–513.
[22] K.H. Assi, W. Tarsin, H. Chrystyn, High performance liquid chromatography assay method for simultaneous quantitation of formoterol and budesonide in Symbicort turbuhaler, J. Pharm. Biomed. Anal. 41 (2006) 325–328.
[23] Y. Mao, P.W. Carr, Separation of selected basic pharmaceuticals by reversed-phase and ion-exchange chromatography using thermally tuned tandem columns, Anal. Chem. 73 (2001) 4478–4485.
[24] D.V. McCalley, Effect of temperature and flow-rate on analysis of basic compounds in high-performance liquid chromatography using a reversed-phase column, J. Chromatogr. A 902 (2000) 311–321.
[25] Validation of Analytical Procedures: Methodology Q2B, ICH Steering Committee, 6 November 1996, website http://www.ich.org/.
[26] I. Apostol, K.J. Miller, J. Ratto, D.N. Kelner, Comparison of different approaches for evaluation of the detection and quantitation limits of a purity method: a case study using a capillary isoelectrofocusing method for a monoclonal antibody, Anal. Biochem. 385 (2009) 101–106.
[27] C. Dunbar, A. Kataya, T. Tiangbe, Reducing bounce effects in the Andersen cascade impactor, Int. J. Pharm. 301 (2005) 25–32.
[28] European Pharmacopoeia, Ph. Eur. (2008) Section 2.9.18.
[29] United States Pharmacopeia, USP 28 (2005) 2359–2377.
[30] W. Tarsin, K.H. Assi, H. Chrystyn, In-vitro intra- and inter-inhaler flow rate-dependent dosage emission from a combination of budesonide and eformoterol in a dry powder inhaler, J. Aerosol. Med. 17 (2004) 25–32.