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On-line high-performance liquid chromatography method for analyte quantitation from pressurized metered dose inhalers

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Abstract

A sensitive and rapid, on-line reversed-phase high-performance liquid chromatographic method for quantitation of compounds at low concentrations in pressurized metered dose inhaler (MDI) systems was developed. Traditional methods for the quantitation of compounds in MDI formulations involve the opening of the MDI vial along with sample dilution prior to quantitation. The new method, reported in this study, involves a direct injection from the MDI vial into the needle injector port of a manual injector. Since there is no dilution step involved, this method can be used to quantitate low concentrations of compounds in MDIs with excellent precision. In addition, since the method requires a small injection volume of 5 μl, repeated analyses can be performed in order to generate multiple data points using the same MDI vial. Validation of the method was performed using ethanol–1,1,1,2-tetrafluoroethane (134a)-based MDIs. Beclomethasone dipropionate (BDP), a corticosteroid used for the treatment of asthma, was used as a model compound. Phase separation studies were conducted to investigate the miscibility of the ethanol–134a mixtures with different mobile phase solvent compositions. For the MDI systems in this study, an acetonitrile–water (90:10, v/v) mobile phase at a flow rate of 0.9 ml/min was found to give acceptable chromatography for BDP on a Apollo C18 5 μm, 150 mm × 4.6 mm column (Alltech Associates, Deerfield, IL, USA). Ultraviolet detection was done at 240 nm and the retention time of BDP was 2.7 min. The on-line HPLC method was characterized to be accurate, precise, sensitive, and specific.

Keywords: Metered dose inhalers; Aerosols; Beclomethasone dipropionate; Tetrafluoroethane; Corticosteroids

1. Introduction

The incidence of asthma has steadily increased in recent decades. In the USA alone about 15 million people suffer from asthma [1–3]. Inhalation is the most frequent route of drug delivery for the effective treatment of asthma while minimizing systemic side effects. Pressurized metered dose inhalers (MDIs) are commonly used to deliver bronchodilators and corticosteroids. MDIs require a propellant such as hydrofluoroalkane (HFA) 134a (1,1,1,2-tetrafluoroethane) or HFA 227 (1,1,1,2,3,3,3-heptafluoropropane) to facilitate the aerosol generation of drug particles. Since 134a (boiling point, −26.1 °C) and 227 (boiling point, −16.5 °C) are gases at room temperature, quantitation of compounds in MDIs using these propellants presents technological challenges. Quantitation of the active ingredient, as well as other constituents in a MDI formulation, is essential for the development and acceptance of a MDI product. The current techniques available for quantitative analysis of compounds in MDI formulations either involve opening a chilled MDI vial and diluting the contents with a suitable mobile phase for analysis [4,5] or by actuating the contents of a MDI vial into a suitable diluent [6]. Compounds present in very low concentrations, whether the active ingredient, degradation products of the active ingredient, excipients or extractables from MDI vial components, are often difficult to analyze due to lack of sensitivity [7,8]. When necessary, compound levels can be increased by combining several different samples in order to achieve an amount that enables quantitation or identification of the compound. However, even when a compound can be detected from a MDI system using traditional methods, precision is often not adequate due to analytical variability of the methods [5]. Hence, there is a need for an alternative analytical method that affords the sensitivity and precision needed for MDI development.

The objective of the present study was to develop a sensitive on-line reversed-phase HPLC method for quantitating low concentrations of analytes in HFA-based MDIs. In the

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current investigation ethanol was used as the cosolvent, since it is the most common cosolvent used for MDIs [9–12]. Beclomethasone dipropionate (BDP), an established corticosteroid used widely for the treatment of asthma [13–17], was utilized as the analyte of interest. A reversed-phase HPLC assay was developed to analyze the BDP MDI formulations and was evaluated using standard analytical indices.

2. Materials and methods

2.1. Materials

The pressure resistant glass aerosol vials, continuous valves, and beclomethasone dipropionate used in this study were provided by 3M Drug Delivery Systems (St. Paul, MN, USA). 1,1,1,2-Tetrafluoroethane (Dymel 134a) and ethanol (200 proof) were obtained from DuPont (Wilmington, DE, USA) and Aaper Alcohol and Chemical Co. (Shelbyville, KN, USA), respectively. All other solvents were HPLC grade and were obtained from Aldrich (Milwaukee, WI, USA) and used as received.

2.2. Sample preparation

Three solution MDIs having BDP concentrations of 0.00250, 0.00484, and 0.00856% (w/w) and ethanol concentrations of 5% (w/w) were prepared, in duplicate, in pressure resistant glass vials. The "cold fill" technique [18,19] was used to fill the vials with 134a. Each of these vials was immediately crimped with continuous valves (3M Drug Delivery Systems) using a small-scale bottle crimper Model 3000B (Aerotech Laboratory Equipment, Maryland, NY, USA).

2.3. Instrument set-up

The instrumentation set-up consisted of a Waters 2695 separations module (Waters, Milford, MA, USA) coupled with a Waters 2487 dual-wavelength absorbance detector. The Waters 2695 separations module was connected with a Rheodyne Model 7725 manual sample injector (Rheodyne, L.P. Rohnert Park, CA, USA). In order to make a direct injection from the MDI vial into the manual injector, the MDI vial was connected with a filtration and injection assembly (Fig. 1). This MDI assembly consisted of the MDI vial with a continuous valve, an adapter which was connected to the stem of the continuous valve, and a 0.2 μm Acrodisc PTFE syringe filter (Pall Gelman Lab., Ann Arbor, MI, USA) which was connected to the stem of the adapter. A no. 22 gauge stainless-steel blunt needle was connected to the stem of the filter. To make a direct injection from the pressurized MDI vial into the manual injector, the needle of the MDI vial assembly was inserted into the needle injector port and the MDI vial was actuated. In order to ensure that the ethanol–134a sample remained as a compressed liquid in the analytical loop, a pressure gauge (Amtek US Gauge Division, Sellersville, PA, USA) and an Alltech adjustable back-pressure regulator (Alltech, Deerfield, IL, USA) were connected in line with the waste outlet of the manual injector.

2.4. Phase separation study

To investigate the miscibility of 134a in different mobile phase compositions a ternary phase separation study was conducted. When initial increments of 134a (less than 10%, w/w) were added to different acetonitrile–water compositions (greater than 50% acetonitrile), an apparent single-phase system was observed. However, as the composition of 134a was increased, a biphasic system was observed. For systems with greater than 50% aqueous component, the addition of 134a always resulted in phase separation. Even though there are miscibility issues between acetonitrile:water and 134a, it was found that any ratio of acetonitrile–water may be used for the mobile phase (from 50:50 to 100:0), provided rapid dilution is achieved.

2.5. HPLC assay

The HPLC assay method used to analyze BDP by direct injection from 134a-based MDI vials utilized an Apollo C18 5 μm, 150 mm × 4.6 mm column, maintained at 30 ± 2 °C. Acetonitrile–water (90:10, v/v) was used as the mobile phase with a flow rate of 0.9 ml/min and an injection volume of 5.0 μl. Ultraviolet detection was done at 240 nm with a total run time of 5 min and the retention time of BDP being 2.7 min. Quantitation was based on peak area, using a standard curve, which was prepared daily. In order to make an injection from a MDI vial, the back-pressure on the sample loop was set at approximately 70 psig (1 psi = 6894.76 Pa).

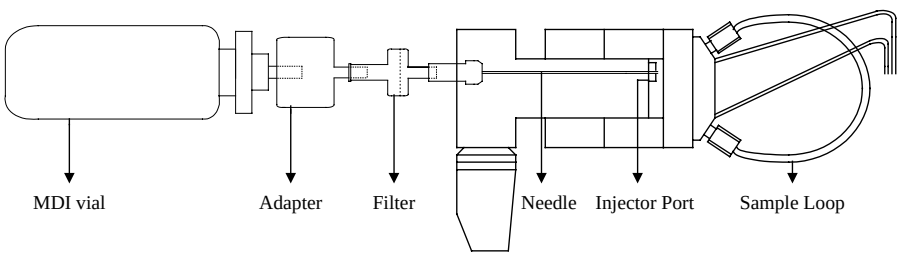

Fig. 1. MDI vial assembly and manual injector.

The MDI vial was actuated and depressed for 3–4 s to fill the sample loop and the injector port was subsequently turned from the 'LOAD' position to the 'INJECT' position. The excess formulation was allowed to discard through the waste line by releasing the back-pressure regulator. The injector port was turned to the 'LOAD' position and the loop was rinsed with 100% acetonitrile. In order to perform the next injection, the back-pressure regulator was adjusted to the initial setting and a new injection was performed in the same manner as mentioned above. Since the sample loop delivers formulations based on volume, a density correction is required. With knowledge of the solvents used for preparing the standards and formulations, the 'mixture densities' were calculated as a linear combination of the constituent solvents and the drug mass dispensed from the sample loop was calculated.

2.6. Preparation of standard solutions for calibration curve and sample evaluation

Stock solutions of BDP (0.032%, w/w) were prepared in 100% acetonitrile and diluted with acetonitrile to obtain a concentration range of 0.001–0.016% (w/w). Based on the density for acetonitrile and a 5 μl loop volume, the corresponding concentration range for the calibration curve was 0.039–0.628 μg/injection. Three standard curves were prepared daily for this concentration range, in order to evaluate the linearity. The peak area of BDP was plotted against BDP concentration to construct the standard curve. Three different MDI formulations of BDP, having concentrations of 0.00250, 0.00484, and 0.00856% (w/w), were evaluated.

3. Results: performance characteristics of the analytical method

The analytical peak of BDP was well resolved from the solvent front. Fig. 2a shows a chromatogram of a blank formulation containing 134a and ethanol, and Fig. 2b shows the chromatogram of a formulation containing BDP. ICH and FDA [20,21] guidelines were taken into consideration while evaluating the analytical method. In order to demonstrate the satisfactory nature of the method, the following protocol was implemented during the development and evaluation.

3.1. System suitability

For the system suitability, six consecutive injections were made with a standard solution. Quantitation was done by area of BDP peak. Table 1 lists the system suitability data for six injections on five different days, along with their standard deviations (S.D.s) and relative standard deviations (R.S.D.s). The R.S.D. over a period of 5 days was 0.75%.

3.2. Sensitivity and limit of detection

The limit of detection and the limit of quantitation (LOD and LOQ, respectively) of the method were determined from

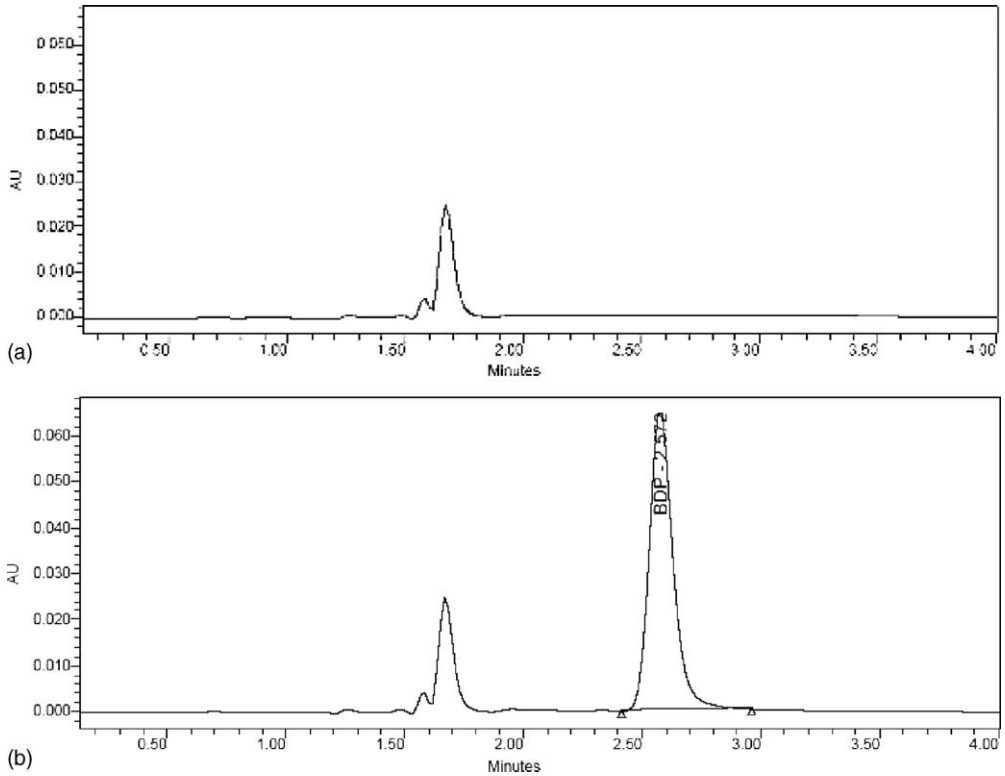

Fig. 2. Representative chromatograms of (a) the propellant system including HFA 134a–ethanol; (b) the formulation containing BDP and HFA 134a–ethanol.

Table 1
Intra-day and inter-day system suitability data for the analytical assay

Day	*n*	AUC	S.D.	R.S.D. (%)	*P* value	Inter-day
1	6	131 000	1419	1.08	1190	
2	6	132 321	1234	0.93	1035	
3	6	133 465	1000	0.75	839	132 412.4[a]
4	6	133 225	2925	2.2	2454	987.7[b]
5	6	132 051	602	0.46	505	0.75[c]

n is the number of injections, AUC is the area under the curve, S.D. is the standard deviation, and R.S.D. is the relative standard deviation.
[a] Mean.
[b] S.D.
[c] R.S.D. (%).

Table 2
Linearity data for the analytical assay

Day	*n*	Slope	S.D.	R.S.D. (%)	95% C.I.	*r*	Inter-day
1	3	1 694 367	9 935	0.59	11 227	0.9999	
2	3	1 685 067	6 806	0.4	7 691	0.9999	
3	3	1 689 467	6 806	0.4	7 691	0.9999	1 690 340[a]
4	3	1 695 567	15 800	0.93	17 854	0.9999	4 521.02[b]
5	3	1 687 233	4 027	0.24	4 551	0.9999	0.27[c]

Calibration curve values were linear on five different days. *n* is the number of injections, slope is the slope of the standard curve prepared daily, S.D. is the standard deviation, 95% C.I. is the 95% confidence interval, *r* is the coefficient of regression, and R.E is the relative error.
[a] Mean.
[b] S.D.
[c] R.S.D. (%).

the standard deviation of response, for known concentrations of BDP. The LOD is defined as the lowest drug concentration, which can be determined and calculated as three times the variation in the measured response [21]. For this method, the LOD was calculated to be 8.83 ng/ml. In the same manner, LOQ was estimated as 10 times the variation in the measured response [21] and was calculated to be 26.75 ng/ml.

3.3. Selectivity and specificity

The selectivity of the developed RP-HPLC method for the determination of BDP in pharmaceutical MDI formulations was investigated at the retention times of the analyte. It is evident from the blank (see Fig. 2) that the propellant and excipient (cosolvent) in the formulation do not cause any interference with BDP. The specificity of the method for BDP was confirmed by library spectra matching. A library spectra confirmed peak purity and the absence of any impurities coeluting with BDP. In addition aliquots of the BDP formulation samples were collected corresponding to the elution time of the BDP peak. The eluates were then combined and analyzed using HPLC–MS (electrospray ionization) in the positive ion mode (LCQ HPLC–MS, Finnigan, San Jose, CA). The analysis confirmed the identity and uniqueness of the BDP peak.

3.4. Linearity

The calibration curve for BDP was prepared in the concentration range of 0.001–0.016% (w/w). This corresponded to 0.039–0.628 μg of BDP per 5 μl injection using 100% acetonitrile. The data for this concentration range were analyzed using least-squares regression analysis: the results are shown in Tables 2 and 3. Linearity was determined by plotting a standard curve from the area of the BDP peak versus the corresponding drug concentration (quantity per injection in μg) in the sample. All the calibration curves were linear on five different days, with a correlation coefficient $r \geq 0.9999$ and with confidence intervals less than $P = 0.05$. The intercepts were not significantly different from zero; therefore, the least-squares regression line was used without an intercept. In addition, the relative error (R.E.) in each concentration was calculated from the calibration curve and ranged from −0.27 to 1.27 (Table 3). The R.E. provides a measure of the difference between the experimental and calculated values and thus a measure of the scatter of the data about the best fit-line.

3.5. Accuracy

Accuracy of the analytical assay was determined as the percentage of the theoretical drug recovered (%T.R., w/w) from the vials containing 0.00250, 0.00484, and 0.00856% (w/w) BDP formulations. The intra-day and inter-day accuracy along with the R.S.D.s and R.E.s are summarized in Table 4. Deviation of the obtained results for the BDP formulations, from the theoretical concentrations were within ±1.43%, during intra-day and inter-day analysis. The *P* values (95% confidence interval) show that the experimental mean was not significantly different from the true value, during intra-day and inter-day analysis.

3.6. Precision

To calculate the precision of the method, intra-day and inter-day tests were performed. The precision was measured in terms of the BDP concentration recovered for the BDP

Table 3
Calibration curve

Sample ($n = 15$)	Drug added (μg)	Drug[a] recovered (μg)	S.D.	R.S.D. (%)	R.E.
1	0.0393	0.0388	0.0005	1.29	1.27
2	0.0786	0.078	0.0009	1.15	0.76
3	0.1571	0.1556	0.0043	2.76	0.95
4	0.3143	0.3119	0.0054	1.73	0.76
5	0.6286	0.6303	0.0037	0.59	−0.27

R.E. is the relative error in each concentration, S.D. is the standard deviation, and R.S.D. is the relative standard deviation.
[a] Calculated using slopes from Table 2.

Table 4
Accuracy data for the analytical assay, for three different drug concentrations

Day	*n*	%T.R.	S.D.	R.S.D. (%)	*P* value	Inter-day
0.00250% (w/w)						
1	6	100.68	0.91	0.91	0.75	
2	6	100.17	0.39	0.39	0.33	
3	6	101.47	1.76	1.73	1.44	101.43[a]
4	6	102.69	2.27	2.21	1.86	1.03[b]
5	6	102.15	2.24	2.19	1.84	1.02[c]
0.00484% (w/w)						
1	6	98.85	2	2.02	1.65	
2	6	100.07	0.88	0.88	0.72	
3	6	100.44	0.25	0.25	0.2	100.47[a]
4	6	102.55	0.54	0.53	0.44	1.34[b]
5	6	100.46	1.75	1.74	1.44	1.33[c]
0.00856% (w/w)						
1	6	100.11	0.79	0.78	0.65	
2	6	101.5	1.63	1.61	1.34	
3	6	102.99	1.43	1.39	1.18	100.79[a]
4	6	99.69	1.03	1.03	0.85	1.44[b]
5	6	99.67	0.65	0.66	0.54	1.43[c]

n is the number of injections, %T.R. is the percentage of the theoretical drug recovered, S.D. is the standard deviation, and R.S.D. is the relative standard deviation.
[a] Mean.
[b] S.D.
[c] R.S.D. (%).

formulations and was expressed as the R.S.D. Intra-day and inter-day variability in the assay was determined by measuring six samples with three different concentrations, for five different days. The values along with S.D.s and R.S.D.s are summarized in Table 5. From the results it is clear that the method is reproducible within the same day and between different days.

4. Discussion

The method described in this report is the first analytical HPLC procedure that enables direct injection from a MDI vial for analyte quantitation. Traditional methods for content determination in MDI systems either involve opening (decrimping) of the MDI vial or actuation of the MDI vial formulation into a suitable solvent [4–6]. This method does not require the physical opening of the MDI vial and does not necessitate the use of several analytical transfer steps. As a result, the method presented offers considerable advantages such as; decreased analysis time, labor and solvent consumption, and increased sensitivity, precision, and repeatability.

The robustness of the current method has been illustrated using BDP as a model compound in ethanol–134a MDI formulations. The United States Pharmacopoeia (USP) requires that the coefficient of variation for the accuracy and precision of the analytical assay used for analysis of the final dosage form, be less than 2.0 [22]. It has been shown through the BDP formulations used in this study, that the on-line HPLC method has the ability to provide a direct analytical means for determining MDI constituents with acceptable accuracy and precision. In addition, the model formulations demonstrate the potential sensitivity of the method (26.75 ng/ml or 0.00044%, w/w) which could be improved through a different detector selection.

Table 5
Precision data for the analytical assay, for three different drug concentrations

Day	*n*	Mean	S.D.	R.S.D. (%)	*P* value	R.E.	Inter-day
0.00250% (w/w)							
1	6	0.00251	2.27×10^{-5}	0.91	1.87×10^{-5}	−0.4	
2	6	0.00250	9.95×10^{-5}	0.39	8.18×10^{-6}	0	0.00253[a]
3	6	0.00254	4.38×10^{-5}	1.73	3.60×10^{-5}	−1.6	2.58E−05[b]
4	6	0.00257	5.65×10^{-5}	2.2	4.64×10^{-5}	−2.8	1.02[c]
5	6	0.00255	5.58×10^{-5}	2.18	4.59×10^{-5}	−2	−1.2[d]
0.00484% (w/w)							
1	6	0.00478	9.68×10^{-5}	2.02	7.96×10^{-5}	1.24	
2	6	0.00484	4.24×10^{-5}	0.88	3.49×10^{-5}	0	0.00486[a]
3	6	0.00486	1.19×10^{-5}	0.25	9.78×10^{-5}	−0.41	6.45E−05[b]
4	6	0.00496	2.61×10^{-5}	0.53	2.15×10^{-5}	−2.48	1.33[c]
5	6	0.00486	8.44×10^{-5}	1.74	6.94×10^{-5}	−0.41	0.41[d]
0.00856% (w/w)							
1	6	0.00857	6.77×10^{-5}	0.78	5.56×10^{-5}	−0.12	
2	6	0.00869	0.00013	1.61	0.000107	−1.52	0.00863[a]
3	6	0.00882	0.00012	1.39	9.86×10^{-5}	−3.04	0.00012[b]
4	6	0.00853	8.79×10^{-5}	1.03	7.23×10^{-5}	0.35	1.43[c]
5	6	0.00853	5.59×10^{-5}	0.66	4.59×10^{-5}	0.35	−0.82[d]

n is the number of injections, mean is the average concentration recovered for six injections, S.D. is the standard deviation, R.S.D. is the relative standard deviation, and R.E. is the relative error.
[a] Mean.
[b] S.D.
[c] R.S.D. (%).
[d] R.E.

The utility of this method can be extended not only to other solution formulations of BDP but for analysis of a variety of compounds in MDI formulations (and propellants), including other active ingredients, degradation products, excipients and possibly extractables. With the right combination of injection volume and detector sensitivity (detector type) it may be possible to analyze low analyte concentrations of compounds that are otherwise difficult to quantitate without concentrating the samples, prior to analysis. While for the current study a relatively high acetonitrile concentration was preferred, giving a k' of 0.633, it is also possible to decrease the acetonitrile concentration to allow better specificity for degradation products, without compromising precision and accuracy. In fact, after the direct injection from the MDI vial is performed, the initial isocratic mobile phase composition can be changed to a gradient method. In summary, the simplicity of the technique, the non-invasive sampling, the minimal volume requirement, and the high sensitivity and precision make this technique particularly attractive for the quantification of compounds in pharmaceutical MDI dosage forms.

References

[1] Vital and Health Statistics, Table 62, vol. 10, Suppl. 193, December 1993.
[2] Survillance for Asthma-United States 1960–1995, Morbidity and Mortality Weekly Report, vol. 47, 24 April 1998, p. SS-1.
[3] HHS Targets Efforts on Asthma, US Department of Health and Human Services, 21 May 1998.
[4] R.O. Williams, J. Liu, Eur. J. Pharm. Biopharm. 47 (1999) 145.
[5] F.E. Blondino, P.R. Byron, J. Pharm. Biomed. 13 (2) (1999) 111.
[6] P.J. Simms, R.W. Towne, C.S. Gross, R.E. Miller, J. Pharm. Biomed. 17 (1998) 841.
[7] S.K. Sethi, D.L. Norwood, P.A. Haywood, D.J. Prime, Biopharm. Sci. 3 (1992) 63.
[8] D.L. Norwood, D. Prime, B.P. Downey, L. Creasey, J.S.K. Sethi, P.J. Haywood, J. Pharm. Biomed. 13 (1995) 293.
[9] R.N. Dalby, A.J. Hickey, S.L. Tiano, in: A.J. Hickey (Ed.), Inhalation Aerosols Physical and Biological Basis for Therapy, Marcel Dekker, New York, 1996.
[10] M.E. Whitham, A.M. Eagle, in: P.R. Byron, R.N. Dalby, S.J. Farr (Eds.), Proceedings of the Respiratory Drug Delivery IV, Interpharm Press, Buffalo Grove, IL, 1994, p. 203.
[11] P.R. Byron, in: P.R. Byron (Ed.), Respiratory Drug Delivery, CRC Press, Boca Raton, FL, 1990, p. 167.
[12] W.F. Kirk, J. Pharm. Sci. 61 (1972) 262.
[13] G. Davies, P. Thomas, I. Broder, et al., Ann. Int. Med. 86 (1977) 549.
[14] J.H. Toogood, N.M. Lefcoe, D.S.M. Haines, et al., J. Allergy Clin. Immunol. 62 (1978) 72.
[15] J.H. Toogood, N.M. Lefcoe, D.S.M. Haines, et al., J. Allergy Clin. Immunol. 59 (1977) 298.
[16] M.H. Williams, Ann. Int. Med. 95 (1981) 464.
[17] M.J. Smith, M.E. Hoodson, Lancet 1 (1983) 265.
[18] M. LeBelle, R.K. Pike, S.J. Graham, E.D. Ormsby, H.A. Bogard, J. Pharm. Biomed. 14 (1996) 793.
[19] A. Gupta, S.W. Stein, P.B. Myrdal, J. Aerosol Med. 16 (2003) 167.
[20] US Food and Drugs Administration (FDA), Guidance for Industry, Analytical Procedures and Methods Validation, August 2000.
[21] ICH Q2B: Validation of Analytical Procedures: Methodology, May 1997.
[22] Physical Tests and Determinations, ⟨6 2 1⟩ chromatography, in: Proceedings of The United States Pharmacopoeia XXII, The United States Pharmacopoeial Convention, Rockville, MD, 1989, p. 1558.