Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 798–803
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Simultaneous, stability indicating, HPLC-DAD determination of guaifenesin and methyl and propyl-parabens in cough syrup

Giorgio Grosa*, Erika Del Grosso, Roberta Russo, Gianna Allegrone

Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università degli Studi del Piemonte Orientale "Amedeo Avogadro", Via Bovio 6, 28100 Novara, Italy

Received 2 September 2005; received in revised form 9 January 2006; accepted 12 January 2006
Available online 23 February 2006

Abstract

A stability indicating high performance liquid chromatography procedure has been developed for the simultaneous determination of guaifenesin (GUA), methyl *p*-hydroxybenzoate (MHB) and propyl *p*-hydroxybenzoate (PHB) in a commercial cough syrup dosage form. The method was specific and stability indicating as chromatographic conditions were selected to provide adequate separation of GUA, MHB and PHB from the putative degradation products guaiacol (GUAI) and *p*-hydroxybenzoic acid (HBA) as well as from excipients. The isocratic separation and quantitation were achieved within 17 min on a 150-mm column with an ether-linked phenyl stationary phase and a hydrophilic endcapping. The mobile phase was constituted of eluant A: aqueous phosphate buffer (pH 3.0, 10 mM)/acetonitrile 25/75 (v/v) and eluant B:methanol; the A:B ratio was 85:15 (v/v) with a flow rate 1 ml min^{-1} and detection of analytes at 254 and 276 nm. The method showed good linearity for the GUA–MHB–PHB mixture in the 95–285, 4–12, and 1–3 μg ml^{-1} ranges, respectively, being all the square of the correlation coefficients greater than 0.999. The interday R.S.D.s were 1.17, 1.14, and 0.91%, for GUA, MHB, and PHP, respectively. The method demonstrated also to be accurate; indeed the average recoveries, at 100% of the target assay concentration, were 100.5, 100.3, and 100.7% with relative standard deviations of 0.8, 0.7, and 0.4% for GUA, MHB, and PHB, respectively, from laboratory prepared samples. The applicability of the method was evaluated in commercial dosage form analysis as well as in stability studies.

Keywords: Guaifenesin; Methyl *p*-hydroxybenzoate; Propyl *p*-hydroxybenzoate; Cough–cold formulation; Syrup; HPLC

1. Introduction

Guaifenesin (GUA), (±)-3-(2-methoxyphenoxy)-propane-1,2-diol, is a widely used expectorant, useful for the symptomatic relief of respiratory conditions (Fig. 1). It is available, alone or in combination with other drugs, mainly as syrup in cough–cold formulations. A preservant system, containing sodium benzoate or combinations of parabens such as methyl *p*-hydroxybenzoate (MHB) and propyl *p*-hydroxybenzoate (PHB) (Fig. 1), was generally used to prevent syrup alteration and degradation.

Several HPLC methods have been reported in literature for the determination of guaifenesin in the presence of other drugs in solid and liquid formulations [1–17]. Moreover some of these methods showed quantitative determination of the preservative sodium benzoate [2,3,7,9–11]. Similarly in current USP monographs [18], GUA was determined by HPLC either individually or in combination with other drugs as pseudoephedrine, codeine, and dextromethorphan.

On the contrary, there was only one method [5] reporting the simultaneous estimation of GUA (in combination with acetaminophen, pseudoephedrine, and folcodine) and the methyl and propyl parabens preservatives in syrup. However, this procedure was not stability indicating as did not account for the presence of guaiacol (GUAI) and 4-hydroxybenzoic acid (HBA) (Fig. 1), the putative degradation products of GUA and parabens, respectively. On the other hand, the preservative assay on stored samples in drug stability studies should be carried out to determine the content of antimicrobial preservatives as indicated by ICH guidelines [19].

* Corresponding author. Tel.: +39 321375854; fax: +39 321375821.
E-mail address: grosa@pharm.unipmn.it (G. Grosa).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.01.026

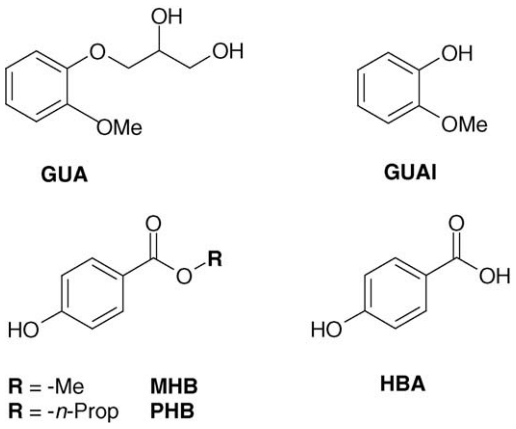

Fig. 1. Structural formulae of guaifenesin (GUA), guaiacol (GUAI), methyl-4-hydroxybenzoate (MHB), propyl-4-hydroxybenzoate (PHB), and 4-hydroxybenzoic acid (HBA).

Hence, in the present work was described a simple, precise, and accurate method for simultaneous estimation of GUA, MHB, and PHB in cough-syrup in the presence of GUAI and HBA. The validation of the proposed method was also carried out and its applicability was evaluated in commercial dosage form analysis.

2. Experimental

2.1. Reagents and chemicals

Methyl *p*-hydroxybenzoate (purity > 99%), propyl *p*-hydroxybenzoate (purity > 99%), *p*-hydroxybenzoic acid (purity > 99%), guaiacol (purity > 98%), methanol, acetonitrile (both HPLC grade), potassium dihydrogen phosphate, concentrated ortophosphoric acid, saccharose, and glycerin were purchased from Sigma–Aldrich (Milano, Italy). Water (HPLC grade) was obtained from Milli-Q RO system.

Broncovanil® syrup (lot: 2001, expiration date: 01/2007), guaifenesin reference standard and anise natural flavour were obtained as gift samples from Pharmafar srl (Torino, Italy).

2.2. Instrumentation and chromatographic conditions

A Shimadzu HPLC system, consisting in two LC-10ADVp module pumps and a DGU-14-A on-line degasser, was used for the analysis. The method was carried out on a Synergi Polar-RP (150 mm × 4.6 mm i.d., 5 μm particle size; Phenomenex, Torrance, USA) column as a stationary phase. The isocratic mobile phase (flow rate 1.0 ml min^{-1}) was composed of eluant A: aqueous phosphate buffer (pH 3.0; 10 mM)/acetonitrile 25/75 (v/v) and eluant B (methanol), the A:B ratio being 85:15 (v/v). The eluant A and B were filtered through a 0.2 μm PTFE membrane filter prior the use. A SIL-10ADVp autosampler was used for the injection of samples (20 μl). The SPD-M10Avp photodiode array detector was used to detect the analytes at 254 (MHB, PHB, and HBA) and 276 nm (GUA and GUAI). A ClassVp 5.03 software was used to process the chromatograms. All the analysis were carried out at room temperature.

2.3. Preparation of standard solutions

2.3.1. Stock solution

A combined standard stock solution of accurately weighted preservatives MHB (400 mg) and PHB (100 mg) was prepared in a 100 ml volumetric flask, using a mixture of water:acetonitrile (75:25, v/v). A mixed standard solution containing MHB, PHB, and GUA was prepared taking into a 500 ml volumetric flask 5.0 ml of the preservatives stock solution and an accurately weighted amount of GUA (475 mg). The resulting mixture was vortexed and made up to 500 ml with water:acetonitrile (75:25, v/v). The final concentrations of analytes were: MHB 40 μg ml^{-1}, PHB 10 μg ml^{-1}, and GUA 950 μg ml^{-1}.

2.3.2. Working standard solution

5.0 ml of the stock solution was further diluted (1:5) with the same solvent to get the following final concentrations of analytes: MHB 8 μg ml^{-1}, PHB 2 μg ml^{-1}, and GUA 190 μg ml^{-1}.

2.4. Preparation of analytical samples

Five millilitre of the Broncovanil® cough-syrup (containing 1.9, 0.080, and 0.020 g of GUA, MHB, and PHB, respectively, in 100 ml) were transferred into a 100 ml volumetric flask, made up to 100 ml with water:acetonitrile (75:25, v/v) and vortexed for 2 min. The resulting solution was further 1:5 diluted using the same solvent to get a concentration of 190 μg ml^{-1} of GUA, 8 μg ml^{-1} of MHB, and 2 μg ml^{-1} of PHB (theoretical values) and this was used, after filtration through 0.2 μm PTFE membrane filter, for the HPLC analysis (sample solution).

2.5. Validation procedure

2.5.1. System suitability

The system suitability parameters, theoretical plates (*N*), and asymmetry factor (As), were calculated, as reported by European Pharmacopoeia [20], with the following formulae:

$$N = 5.54\left(\frac{t_R}{W_h}\right)^2, \qquad As = \frac{W_{0.005}}{2d}$$

where t_R is the retention time of the component, W_h the width of the peak at half-height, $W_{0.05}$ the width of the peak at one-twentieth of the peak height, and *d* is the distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

2.5.2. Specificity

To assess the method specificity, a reconstituted syrup without guaifenesin and parabens was prepared and aromatized with the excipients as for commercial preparation of Broncovanil®. For HPLC analysis, the solution was prepared using the same procedure of analytical sample. Moreover to evaluate the influence of the putative degradation products GUAI and HBA and resolution factors, a standard stock solution was prepared as reported above, except the addition of GUAI and HBA at 47.5 and 0.5 μg ml^{-1} concentrations, respectively. HPLC analysis

were performed after dilution as reported for working standard solution. Resolution factors were calculated with the following formula:

$$\mathrm{Rs} = 1.18\frac{t_{R2} - t_{R1}}{(W_h)2 + (W_h)1}$$

2.5.3. Linearity

Linearity of the method was evaluated at five equispaced concentration levels by diluting the standard stock solution to give solutions at 150, 125, 100, 75, and 50% of the target assay concentration. These were injected in triplicate and the peak areas were inputted into a Microsoft Excel spreadsheet program to plot calibration curves.

In order to satisfy basic requirements such as homoscedasticity and linearity, the Bartlett test and the lack-of-fit tests were, respectively, performed at the 95% significance level.

2.5.4. Precision

Precision was evaluated in terms of intraday and interday precision.

The intraday precision was investigated using six separate sample solutions prepared, as reported above, from a freshly reconstituted syrup formulation at 100% of the target level. Each solution was injected in triplicate and the peak areas obtained were used to calculate mean and %R.S.D. values.

The interday precision was checked on six different days, by preparing and analysing in triplicate six separate sample solutions from the reconstituted syrup at the same concentration level of intraday precision; the means and %R.S.D. were calculated from peak areas.

2.5.5. Accuracy

To assess accuracy, a freshly prepared placebo syrup was spiked with various amounts of GUA, MHB, and PHB at 80, 100, and 120% of the target concentrations.

For HPLC analysis, the solutions were prepared as reported for analytical sample; each solution was injected in triplicate and the peak areas were used to calculate mean and %R.S.D. values and compared with those obtained with standard solution.

3. Results and discussion

3.1. Method development

The aim of this study was to develop a single isocratic HPLC assay for the simultaneous analysis of GUA, MHB, and PHB in presence of their putative degradation products GUAI and HBA. The task was difficult because the analytes have different lipophilic characters: indeed logP(oct/w) of PHB and HBA were 3.04 and 1.58 [21], respectively, with the presence in the HBA structure of an ionizable carboxylic function (pKa 4.54). Initial studies to develop the method involved the use of C-18, C-8, and phenyl-reverse phase columns with various mobile phases containing acetonitrile or methanol-aqueous phosphate buffers. In almost every system studied, while the separation of more polar compounds was in some instance obtained, PHB showed a retention time greater than 20 min. Furthermore, in some instance GUA and HBA were co-eluted (data not shown).

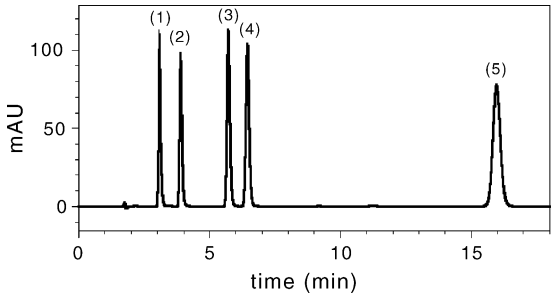

Fig. 2. HPLC chromatogram of HBA (1) GUA (2), GUAI (3), MHB (4), and PHB (5) reference standards. UV detection at 254 nm.

To ensure greater retention time to polar analytes without increasing total runtime, an ether-linked phenyl stationary phase with a hydrophilic endcapping was used.

In this case, the optimized mobile phase was constituted by eluant A:aqueous phosphate buffer (pH 3.0, 10 mM):acetonitrile 25:75 (v/v) and eluant B:methanol, the A:B ratio being 85:15 and the flow rate 1 ml min^{-1}. The addition of methanol enhanced aromatic selectivity because the π–π interactions between aromatic rings of the analyte and the phenyl functional group of the stationary phase were favoured; moreover an acidic phosphate buffer was employed to suppress the ionization of HBA carboxylic function. Taken together these features provided chromatograms with good peak shape with a steady baseline required for the simultaneous analysis of a GUA–MHB–PHB–GUAI–HBA mixture with an acceptable runtime (Fig. 2). The retention factors k were 1.1, 1.6, 2.9, 3.3, 9.7 for HBA, GUA, GUAI, MHB, and PHB, respectively, being the range within $1 < k < 10$. Generally, to obtain the best chromatographic behavior for isocratic separations, $2 < k < 10$ was desired [22]; however, when the analytes showed great differences in polarity, the range $1 < k < 20$ was, in some instance, acceptable. For quantitative determination, detection of GUA and GUAI was performed at 276 nm; however, at this wavelenght MHB, PHB, and HBA showed poor absorbance properties hence they were detected at 254 nm.

3.2. Method validation

The developed method was validated, as described below, for the following parameters: system suitability, specificity, linearity, precision, accuracy, and LOD.

3.2.1. System suitability

As system suitability test was an integral part of chromatographic methods developmemt and were used to verify that the system is adequate for the analysis to be performed, the parameters for GUA, MHB, and PHB were evaluated. The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values, reported in Table 1, with the acceptance criteria of the CDER guidance document [23].

Table 1
System suitability parameters

	GUA	MHB	PHB	CDER acceptance criteria
Asymmetry	1.2 (2.3)	1.1 (2.3)	1.1 (1.5)	<2.0
Theoretical plates	8878 (1.9)	12253 (2.1)	13794 (1.7)	>2000
Repeatability of peak area	231.9856 (0.4)	92.5987 (0.6)	19.5891 (0.6)	<1.0%

The concentration of GUA, MHB, and PHB were 190, 8, and 2 μg ml^{-1}, respectively. The figures in parenthesis represent %R.S.D. values for six replicates.

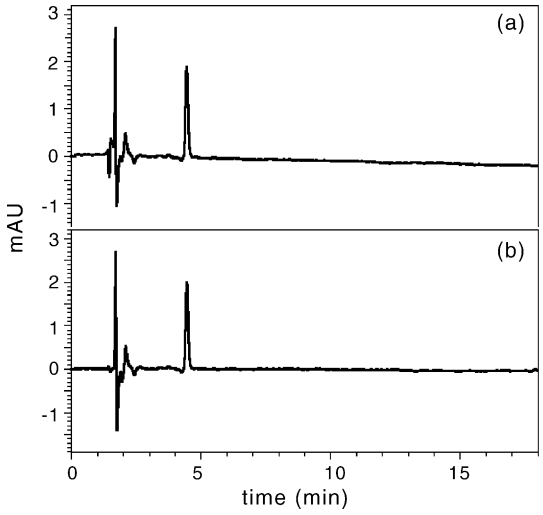

Fig. 3. HPLC chromatograms of (a) placebo and (b) anise flavour. UV detection at 254 nm.

3.2.2. Specificity

Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of components that are present in the sample matrix. The representative chromatogram (Fig. 3a) of placebo solution constituted by excipient blend showed only one peak which was referred to anethol, the main component of anise flavour [24] (Fig. 3b); however, it appeared well separated from GUAI peak (Fig. 4).

Indeed the resolution factors, calculated for adjacent peaks GUAI/anise flavour, HBA/GUAI, and GUA/MHB were 3.9, 4.8, and 2.9, respectively. Moreover, the resolution values calculated at different time-points over a 36 month stability protocol did not show significant differences. In particular, the resolution values of the more critical couple of adjacent peaks (GUA/MHB) were always within the range 2.9–3.0. These data demonstrated that excipients and putative degradation products did not interfer with the drug and preservatives peaks indicating specificity of the method.

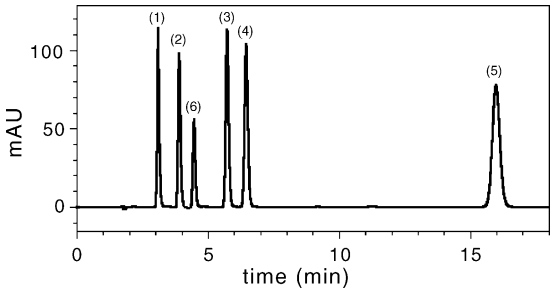

Fig. 4. HPLC chromatograms of a combined solution of anise natural flavour standard (6) and HBA (1) GUAI (2), GUA (3), MHB (4), and PHB (5) standards. UV detection at 254 nm.

3.2.3. Linearity

The linearity of an analytical procedure is its ability, within a given range, to obtain test results which are directly, or through a mathematical transformation, proportional to the concentration of analyte. Five equispaced concentration levels within 50–150% of the target concentration range were considered to study the linearity. Since the Bartlett test evidenced no significant difference ($p > 0.05$) among the variance values of replicates at different concentration levels the best fit was obtained using an unweighted linear regression model. The linearity was observed in the expected concentration range, demonstrating its suitability for analysis.

The results of the regression statistic obtained for GUA, MHB, and PHB were reported in Table 2. The square of the correlation coefficients ($r^2 \geq 0.999$) demonstrated a significant correlation between the concentration of analytes and detector response; however, the coefficient of correlation was neither a proof of linearity, nor a useful measure of the calibration variability.

Hence, the lack-of-fit-test were performed on these data; the significance values ($p > 0.05$) obtained for all analytes indicated that a linear regression model provides a good interpolation of the experimental data.

Table 2
Five levels calibration graphs of GUA, MHB, and PHB: unweighted linear regression $y = ax + b$; three replicates for each level ($n = 15$)

Analyte	Concentration (μg ml^{-1})[a]	Range (μg ml^{-1})	a (S.E.)[b]	b	b CI[c]	r^2	%R.R.S.D.[d]
GUA	190	95–285	1215.5 (9.981)	0.17075	−4.175 to 4.517	0.9991	1.06
MHB	8	4–12	11.497 (01081)	0.33851	−2.320 to 1.643	0.9990	1.28
PHB	2	1–3	9.7289 (0.0831)	0.10162	−0.282 to 0.479	0.9991	1.17

[a] Target concentration corresponding to 100%.
[b] Standard error.
[c] 95% Confidence interval.
[d] %Relative residual standard deviation.

Table 3
Intraday and interday precision data for GUA, MHB, and PHB

Analyte	% of target concentratior	Intraday variation (%R.S.D.)	Interday variation (%R.S.D.)
GUA	100[a]	0.60 ($n = 12$)	0.92 ($n = 18$)
MHB	100[a]	0.61 ($n = 12$)	0.87 ($n = 18$)
PHB	100[a]	0.67 ($n = 12$)	0.80 ($n = 18$)

[a] Hundred percent of target concentration is equivalent to 190 μg/ml of guaifenesin, 8 μg/ml of MHB, and 2 μg/ml of PHB.

Moreover, the evaluation of residual plot confirmed that underlying assumption like homoscedasticity was met as well as the goodness of fit of the regression model.

Finally the confidence interval of the *y*-intercepts includes zero and the relative residual standard deviations, expressed as percentage, were calculated and used to assess the precision of the regression; the obtained values were lower than 1.5%.

3.2.4. Precision

The precision of an analytical procedure expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The intraday precision refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment.

Interday precision involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts. The results obtained are shown in Table 3. In all instances, the %R.S.D. values were less than 2%.

3.2.5. Accuracy

The accuracy of an analytical method is the closeness of the test results to the true value. It has been determined by application of the analytical procedure to recovery studies, where known amount of standard is spiked in the placebo. The results of accuracy studies from standard solution and excipient matrix were shown in Table 4; recovery values demonstrated that the method was accurate within the desired range.

3.2.6. LOD

The LOD of an analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated. It is expressed as a concentration at a specified signal-to-noise ratio, usually three. The limit of detection was determined, by injecting progressively low concentration, only

Table 4
Accuracy: recovery data for GUA, MHB and PHB

% of targeting concentration[a]	GUA % recovery	MHB % recovery	PHB % recovery
80	100.73 (0.34)	100.62 (0.24)	99.77 (0.42)
100	100.49 (0.84)	100.3 (0.67)	100.71 (0.37)
120	99.97 (0.65)	99.96 (0.73)	100.61 (1.20)
Average recovery	100.4 (0.61)	100.29 (0.55)	100.37 (0.66)

[a] Hundred percent of target concentration is equivalent to 190 μg/ml of guaifenesin, 8 μg/ml of MHB, and 2 μg/ml of PHB. The figures in parenthesis represent %R.S.D. values for three replicates.

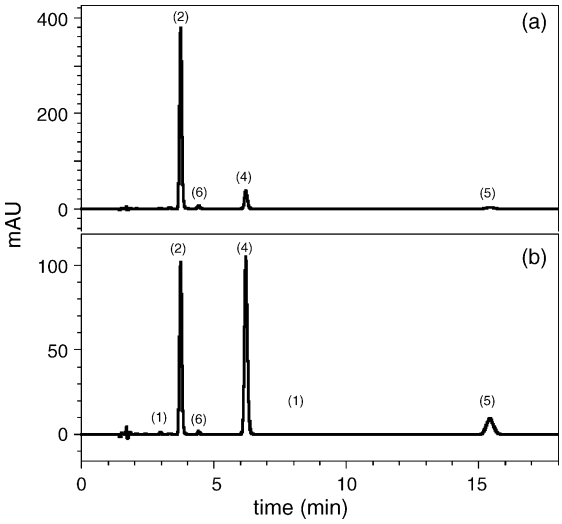

Fig. 5. HPLC chromatograms of a syrup sample after 24 months stability test at (a) 276 and (b) 254 nm UV detection.

for the putative degradation products GUAI and HBA: the concentrations were 0.022 and 1.3 μg ml^{-1} for HBA and GUAI, respectively.

3.3. Analysis of a commercial syrup formulation

The chromatograms in Fig. 5 were obtained using the described HPLC method with a syrup sample arising from a long-term stability study stored 24 months in original packaging (temperature 25 ± 2 °C and relative humidity 60 ± 5%). As expected, GUA at 276 nm (Fig. 5a), and both preservatives, MHB and PHB as well as the excipient anise flavour at 254 nm (Fig. 5b) were detected and appeared well separated. Moreover, the HPLC trace at 276 nm allowed to exclude the formation of GUAI the putative degradation product of GUA. However, at 254 nm (Fig. 5b) a low intensity peak referable to HBA was observed at 3.0 min. Indeed in aqueous liquid formulation hydrolysis of ester function should be a concern during the stability study.

4. Conclusions

The analytical results demonstrated the ability of the developed HPLC method to simultaneous assay GUA and paraben preservatives MHB and PHB in the presence of their putative degradation products. The complete separation of the analytes was accomplished isocratically in only 17 min. The method has been successfully used to perform long-term and accelerate stability studies of GUA syrup formulation.

References

[1] V.D. Gupta, A.G. Ghanekar, J. Pharm. Sci. 66 (1977) 895–897.
[2] D.R. Heidemann, J. Pharm. Sci. 68 (1979) 530–532.
[3] N. Muhammad, J.A. Bodnar, J. Liq. Chromatogr. 3 (1980) 113–122.
[4] W.O. McSharry, I.V.E. Savage, J. Pharm. Sci. 69 (1980) 212–214.
[5] L. Carnevale, J. Pharm. Sci. 72 (1983) 196–198.

[6] G.W. Schieffer, D.E. Hughes, J. Pharm. Sci. 72 (1983) 55–59.
[7] G.W. Schieffer, W.O. Smith, G.S. Lubey, D.G. Newby, J. Pharm. Sci. 73 (1984) 1856–1858.
[8] D.R. Heidemann, K.S. Groon, J.M. Smith, LCGC 5 (1987) 422–426.
[9] T. Chen, J.R. Pacifico, R.E. Daly, J. Chromatogr. Sci. 26 (1988) 636–639.
[10] T.D. Wilson, W.G. Jump, W.C. Neumann, T. San Martin, J. Chromatogr. 641 (1993) 241–248.
[11] I.I. Hewala, Anal. Lett. 27 (1994) 71–93.
[12] G. Indrayanto, A. Sunarto, Y. Adriani, J. Pharm. Biomed. Anal. 13 (1995) 1555–1559.
[13] J. Holmalahti, Pharmazie 52 (1997) 640–641.
[14] L.A. Shervington, Anal. Lett. 30 (1997) 927–944.
[15] S. Sinan, A. Cemal, C. Semsettin, Farmaco 54 (1999) 705–709.
[16] M.L. Wilcox, J.T. Stewart, J. Pharm. Biomed. Anal. 23 (2000) 909–916.
[17] M. Vasudevan, S. Ravisankar, A. Sathiyanarayanan, R.S. Chandan, J. Pharm. Biomed. Anal. 24 (2000) 25–31.
[18] The United States Pharmacopoeia, 28th ed., The United States Pharmacopeial Convention, Inc., Rockville, MD, 2005, pp. 922–929.
[19] Stability Testing of New Drug Substances and Products, ICH Harmonised Tripartite Guideline, 1995, pp. 1–14.
[20] European Pharmacopoeia, 5.0 ed., Council of Europe, Strasbourg, 2005, pp. 69–72.
[21] C. Hansch, D. Hoekman, A. Leo, L. Zhang, P. Li Toxicol. Let. 79 (1995) 45–53.
[22] J.W. Dolan, LC–GC Eur. 17 (2004) 624–628.
[23] Center for Drug Evaluation and Research, U.S. Food and Drug Administration. Reviewer Guidance, Validation of Chromatographic Methods; FDA, Rockville, MD, November 1994.
[24] M. Puertas-Mejýa, S. Hillebrand, E. Stashenko, P. Winterhalter, Flavour Fragrance J. 17 (2002) 380–384.