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Validation of a HPLC quantification of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations: capsules and sachets

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Abstract

Acetaminophen, phenylephrine and chlorpheniramine are frequently associated in pharmaceutical formulations against the common cold. Their quantification presents several problems. A HPLC method for the simultaneous determination of these compounds in pharmaceutical formulations such as capsules and sachets, including the separation of impurities and excipients has been developed and validated. The selectivity of the method was also tested to be used if phenylpropanolamine hydrochloride were employed instead of phenylephrine. Final chromatographic conditions were a gradient elution, being solvent A: phosphate buffer 40 mM at pH 6.0 and solvent B: acetonitrile. At $t = 0$, the mobile phase consisted of 92% A and 8% B and it changed with a linear gradient during 8 min to 75% A and 25% B. At min 8, it changed to 30% A and 70% B for 5 min and at $t = 15$ min, it returns to the initial conditions (92% A and 8% B) during 1 min remaining at this composition until $t = 20$ min. UV detection was performed at 215 nm for phenylephrine and chlorpheniramine, because at this wavelength sensitivity was higher than in other more characteristic wavelengths and it was necessary for the detection of minor compounds. For acetaminophen 280 nm was employed. Validation parameters permit to consider the method adequate.

Keywords: HPLC; Acetaminophen; Phenylephrine; Chlorpheniramine; Impurities

1. Introduction

Acetaminophen (paracetamol) is analgesic and antipiretic. Phenylephrine is sympathomimetic (descongestants) and chlorpheniramine maleate is an H_1-receptor antagonist (antihistaminic). These substances are frequently associated in pharmaceutical formulations against the common cold, but with an important imbalance between the different actives in the dosage forms. Moreover, the active compounds have very different polarity and, therefore, chromatographic behavior. In some formulations phenylpropanolamine hydrochloride replaces phenylephrine and the problem of their analysis is further complicated by the

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presence of impurities such as 4-aminophenol and 4-chloroacetanilide related to acetaminophen. The dosage forms also contain excipients, some of which may interfere with the analysis of the active ingredients, mainly in the case of flavors in the dispersable sachets and sugar which adsorbs other compounds. No single method is reported to determine the active ingredients quantitatively in this combination.

Table 1 shows a review of the analytical methods published for measuring some of these substances, alone or in different combinations. It includes analytical conditions and some validation parameters when they are described. Phenylpropanolamine hydrochloride and acetaminophen have been determined in pharmaceutical preparations by Raman spectroscopy [1]; Phenylpropanolamine and chlorpheniramine together with other actives have been measured by GC [2–4] and by HPLC [5]. Different analgesic including acetaminophen and chlorpheniramine have been analyzed by HPLC with on-line post-column photochemical derivatization [6] and by GC [7]. Acetaminophen and chlorpheniramine in human plasma have been determined by LC-MS-MS and by HPLC in combination with codeine [8] and pheudoephedrine [9,10]. Paracetamol, phenylpropanolamine and chlorpheniramine and dextromethorphan were separated by MECC [11,12] in cold medicines. Paracetamol and chlorpheniramine has been determined by EKC employing bile salts in cold medicines[13].

Three methods in literature report the quantitation of acetaminophen, chlorpheniramine and phenylpropanolamine. The one by Gupta et al. [14] requires three different analysis with three different mobile phases. Meanwhile, Indrayanto et al. [15] and Zhao et al. [16] developed a simultaneous assay of the three actives by HPLC. Krieger et al. developed a method for the separation of acetaminophen in analgesic preparations containing chlorpheniramine maleate, phenylephrine hydrochloride, and other active components by HPLC [17] but the method did not permit the separation of the impurities, although many modifications were tested Some references of methods developed by column suppliers for several standards have also been included [18–22]. Nevertheless none of them separates the three actives here proposed, and the impurities.

The aim of the present work was the development and validation, following ICH guidelines [23] of a HPLC method for the simultaneous determination of acetaminophen, phenylephrine and chlorpheniramine in pharmaceutical formulations such as capsules and sachets, including the separation of impurities and excipients. The selectivity of the method was also tested to be used if phenylpropanolamine hydrochloride were employed instead of phenylephrine. The chemical structures of the assayed compounds and most of their values for the acid-base constants are shown in Table 2.

2. Experimental

2.1. Apparatus

A Beckman (Palo Alto, USA) HPLC system provided with a 116 pump, an automatic injector (507e), a 166 UV detector and a Gold System data processor were used. The chromatographic analysis were performed on a 5 μm particle SymmetryShield RP8 (Waters, Madrid, Spain) column (250×4.6 mm) kept in a Thermoquest (Madrid, Spain) Gecko 2000 column oven at 35 °C.

Final chromatographic conditions were a gradient elution, being solvent A: phosphate buffer 40 mM at pH 6.0 and solvent B: acetonitrile. The phosphate buffer was prepared from KH_2PO_4 by adding KOH to reach the pH 6.0. At $t = 0$ the mobile phase consisted of 92% A and 8% B and it changed with a linear gradient during 8 min to 75% A and 25% B. At min 8 it changed to 30% A and 70% B for 5 min and at $t = 15$ min it returns to the initial conditions (92% A and 8% B) during 1 min remaining at this composition until $t = 20$ min. The flow rate was 1 ml/min and the injection volume was 20 μl. UV detection was performed at 215 nm for phenylephrine and chlorpheniramine, because at this wavelength sensitivity was higher than in other more characteristic wavelengths and it was necessary for the detection of minor compounds. For acetaminophen 280 nm was employed.

Table 1
Review of the analytical methods for the determination of acetaminophen, phenylephrine, phenylpropanolamine and chlorpheniramine

Compounds	Sample	Technique	Conditions	Notes	Reference
Acetaminophen, chlorpheniramine Maleate, Dextromethorphan HBr, Phenylpropanolamine HCl	Pharmaceutical dosage forms	HPLC	MP: (1) $AcNH_4$ 0.02 M-15% MeOH (v/v) pH 7. (2) KH_2PO_4 0.02 M pH 2.6 with H_3PO_4. (3) Sodium 1-Heptanesulphonate 0.005 M pH 3.3, HAc 1% (v/v), 48% (v/v) MeOH. (4) 1% (v/v) amonium formate buffer pH 4.1–40% MeOH (v/v) IV: 20 μl λ: 262 y 279 nm	R.S.D. (%): 1.2, 2.4, 1.9 and 1.6. Range: Ac: 0.2–0.6, pH 5–15 μg. TA: 12 min	[14]
Phenylpropanolamine HCl, Caffeine, Acetaminophen, Glycerylguaiacolate, Chlorpheniramine maleate	Silabat tablet	HPLC	Column: LiChrospher CN (5 μm, 100 Å). MP: 87% Ion pair pH 3.3 (hexanesulphonic acid sodium salt 5 mM, di-*n*-butylamine 10 mM, acetic acid glacial 0.8% v/v and phosphoric acid 0.12% v/v)-8% THF- 7% Acetonitrile. Flow: 1.0 ml/min. λ: 260, 298, 310, 284 and 265 nm. IV: 20 μl	Recovery (%): 98.5–101.8. R.S.D. (%): 1.12, 1.11, 1.09, 1.07 and 1.30	[15]
Paracetamol, Caffeine, Chlorpheniramine, Phenylpropanolamine HCl	Ganmaoling capsules	HPLC	Column: Spheri-5, RP-18 column (220 × 4.6 mm,5 mm). MP: Acetonitrile–water–Na pentane sulfonate (14 mmol/l)-H_3PO_4 (1 M) (18:82:4:0.5). λ: 210 nm	Range: 2010–4020, 202–404, 23.44–46.88, 43.2–86.4 μg/ml. Recovery: 100.9, 97.9, 97.48 and 97.8. R.S.D. (%): 0.68, 0.32, 0.32 and 0.50	[16]
Acetaminophen, Chlorpheniramine Maleate, Phenylephrine HCl, Caffeine, Salicylamide, Aspirin, Phenacetin, Salicylic acid	Commercial multicomponent analgesic preparations	HPLC (photometric detection)	Column: Chromegabond C18 (300 × 4.6 mm, 10 μm). MP: Methanol-0.75% acetic acid (1 + 3) pH 3. Flow: 2.0 ml/min. λ: 254 and 280 nm. IV: 20 μl	Recovery (%): 101.9. R.S.D. (%): 0.29–1.01. TA: 28 min. Range: 1–500 μg/ml. Tailing factor: <2	[17]

Table 1 (*Continued*)

Compounds	Sample	Technique	Conditions	Notes	Reference
Paracetamol, Pseudoephedrine, Chlorpheniramine	Cold relief ingredients in chewing gum	HPLC	Column: Spherisorb C18 (250 × 4.6 mm, 5 μm). MP: Hexylamine 4.5 mM (pH 3)-acetonitrile (88:12 v/v). Flow: 1.0 ml/min. T^a Column: 40 °C. λ: 220 nm. IV: 20 μl	Range, 10–500; 20–100; and 1–30 μg/ml. R.S.D. (%) ($n = 6$), 0.40; 1.1; and 6.4. k, 1.11; 0.41; and 0.94. N, 7273; 2266; and 38 573. ASF, 1.1; 1.8; and 1.0. LOD, 0.03; 0.07; and 0.02 ng. TA, 10 min	[9]
Acetaminophen, Pseudoephedrine HCl, Chlorpheniramine maleate	Cold tablet	HPLC	Column: Zorbax CN C18 (250 × 4.2 mm, 10 μm), MP: 18.0 g Brij 35 in ca. 500 ml of water (50 °C) + 3.46 g SDS disolved in 1 l of water containind 4.5 (%) (v/v) n-propanol pH 2.25 (H_3PO_4). Flow: 1.5 ml/min. T^a Column: 65 °C. λ: 310 and 220 nm. IV: 20 μl	R.S.D. (%), 1.43; 0.59; and 1.34. N, 940; 1380; and 1600. K, 1.6; 5.6; and 7.4. TA, 15 min	[10]
Acetylsalicylic acid, Acetaminophen, Propyphenazone, Caffeine, Chlorpheniramine	Analgesic dosage forms	HPLC (photochemical derivatization)	(1) Column: Spherisorb-CN (150 × 4.6 mm, 5μm); MP: TEA Phosphate 0.1 M pH 3.0—ACN (95:5) (1 ml/min). (2) Column: Hypersil ODS (150 × 4.6 mm, 5μm); MP: THF Phosphate 0.05 M pH 3.0—ACN (1 ml/min). (3) Column: Hypersil ODS (150 × 3.2 mm, 5 μm); MP: THF-TEA Phosphate 0.05 M pH 3.0—ACN (88: 12, v/v) (0.4–0.6 ml/min); T^a Column: ambient; λ: 275 nm; IV: 10 μl	Recovery (%): 98–99.4. R.S.D. (%): 0.7–1.6. Range: 200–1000 (acetaminophen), 50–100 (caffeine), 10–50 (chlorpheniramine) μg/ml	[6]
Phenylpropanolamine, Chlorpheniramine	Standards	HPLC	Column: StableBond SB-CN (150 × 4.6 mm, 5 μm). T^a Column: 35 °C. MP: 80% Na_2HPO_4 25 mM adj. to pH 3 with H_3PO_4-20% MeOH. Flow: 1 ml/min. λ: 254 nm	TA: 15 min	[18]

Table 1 (*Continued*)

Compounds	Sample	Technique	Conditions	Notes	Reference
Phenylpropanolamine HCl, Aspartame, 'Flavors', Chlorpheniramine maleate, Aspirin, Salicylic acid, Dextromethorphan HBR	Standards	HPLC	Column: Maxil C18 (250×4.6 mm, 5 μm, 65 Å). MP: 75% Amonium phosphate buffer 0.1 M pH 2.5–25% ACN. Flow: 0.85 ml/min. λ: 215 nm	TA: 30 min	[19]
Acetaminophen, Hidroclorotiazida, Phenylpropanolamine HCl	Tablet	HPLC	Column: Zorbax Rx C18 (150×4.6 mm, 5 μm). T^a Column: 40 °C. MP: 4% Buffer (Potasium phosphate 25 mM-H_3PO_4 25 mM)-96% CAN. Flow: 1 ml/min. λ: 254 y 205 nm. IV: 20 μl	TA: 5 min. Range: 0.009–0.075 mg/ml	[25]
Maleate, Phenylephrine, Phenylpropanolamine, Pryilamine, Chlorpheniramine	Standards	HPLC	Column: Zorbax SB-Aq (150×4.6 mm, 5 μm). T^a Column: 25 °C. MP: 90% TFA 0.2–10% CAN. Flow: 1.5 ml/min. λ: 254 nm	TA: 16 min	[20]
Phenylpropanolamine, Ephedrine, Amphetamine, Methamphetamine, Phenteramine	Standards	HPLC	Column: Eclipse XDB-C8 (150×4.6 mm, 5 μm). T^a Column: 35 °C. MP: 85% Phosphate buffer 25 mM pH 3–10% ACN. Flow: 1 ml/min	TA: 6 min	[21]
Phenylephrine, Phenylpropanolamine, Pseudoephedrine, Methylparaben	Standards	HPLC	Column: Synergi Polar-RP (150×4.6 mm, 4 μm, 80 Å). T^a Column: Ambient. MP: 65% Potasium phosphate 20 mM pH 6.5–35% Methanol. Flow: 1.5 ml/min. λ: 210 nm. IV: 1 μl	TA: 9 min	[22]
Codeine phosphate, Pseudoephedrine HCl, Chlorpheniramine maleate	Pharmaceutical preparation	HPLC	Column: Waters Symmetry C8 (150×3.9 mm, 5 μm). MP: Ion Pair (2 g octanesulphonic acid sodium salt (PIC-B8)+0.5 ml H_3PO_4 85%/1 l H_2O pH 3.0)-Acetonitrile (gradient). Flow: 1.0 ml/min. T^a Column: 40 °C. λ, 210; 205; and 223 nm. IV, 10 μl		[26]

Table 1 (*Continued*)

Compounds	Sample	Technique	Conditions	Notes	Reference
Pseudoephedrine HCl, Chlorpheniramine maleate, Dextromethorphan HBr	Tablet formulation	HPLC Second-derivative photodiode array spectroscopy	Column: DuPont Zorbax SCX (250×4.6 mm). T^a Column: Ambient. MP: 50% Potassium phophate 0.03 M-50% Acetonitrile. Flow: 2.0 ml/min. λ: 263 nm. IV: 50 μl	RT (min), 10.0; 16.0; and 13.4	[27]
(a) Phenylpropanolamine HCl, Chlorpheniramine maleate. (b) Pseudoephedrine HCl, Chlorpheniramine maleate. (c) Phenylpropanolamine HCl, Pheniramine maleate, Pyrilamine	Pharmaceutical preparation (tablet and syrup)	HPLC	Column: Cation exchange SCX (0.5 m, 6.35 mm o.d, 2.10 mm i.d). MP: $(NH_4)_2PO_4$ 0.02 M in 28–36% dioxane-water (v/v) pH 8.2. Flow: 1.1 ml/min. T^a Column: ambient. IV: 8 μl	TA: 25 min. R.S.D. (%): 1.11–3.24%	[5]
Acetaminophen, Caffeine, Ascorbic acid, Phenylephrine HCl	Pharmaceutical formulations	HPLC [28]	Column: LiChrospher RP-18 (250×4.6 mm, 5 μm)		
3-Aminophenol, Acetaminophen, 3-Hidroxiacetanilida (from Aspirin), Caffeine	Tablet formulation	HPLC	Column: Zorbax ODS (250×4.6 mm, 5 μm). MP: 70% Phosphate buffer (17.7 g KH_2PO_4/0.7 l) pH 3.6–30% Methanol. Flow: 1.0 ml/min. λ: 240 nm. IV: 10 μl	TA: 10 min. R.S.D. (%): 1.8 (Aspirin), 1.3 (Acetaminophen) y 2.1 (Caffeine)	[29]
Chlorpheniramine maleate, Codeine, Acetaminophen, 4-Aminophenol	Codeine in acetaminophen with codeine combination products	HPLC	Column: LiChrosorb Si-60 (250×2.1 mm, 10 μm). MP, 23.2% Methylene chloride; 4.4% Methanol; 72.3% *n*-hexano; and 0.1% Ammonium hydroxide. Flow: 3.0 ml/min. λ: 254 nm	TA: <10 min. R.S.D. (%), 0.5; 0.4; and 0.5. RT, 1.88; 3.82; 6.00; and 3.26 min. LOD: 0.01 (4-Aminophenol). Recovery (%): 100.0–101.2	[8]
Acetaminophen, Chlorpheniramine	Human plasma	LC-MS-MS	Column: Kromasil C18 (50×4.6 mm, 5 μm). MP:H_2O-ACN (80:20), 0.5% formic acid and 1 mM pentafluoropropionic anydride. Flow: 1 ml/min. Split: 200 μl/min. Turboion spray T^a: 350 °C (N_2 7 l/min). Nebulizer gas: air (90 psi). Ionspray voltage: 4000 V	LOQ: 0.5 μg Acet per ml plasma and 0.2 ng Chlor per ml plasma. Sample range: 0.5–25 μg Par per ml and 0.2–50 ng Clor per ml plasma. Working range: 500–1 μg Acet per ml and 1000–2 ng Chlor per ml. R.S.D. (%): 4.60–10.06 (Acet) and 3.21–10.58 (Chlor). TA: 4 min	[30]

Table 1 (*Continued*)

Compounds	Sample	Technique	Conditions	Notes	Reference
Paracetamol, Caffeine, Chlorphenamine maleate	Suxiaoshangfeng capsule	GC	Column: 5% Ph–Me–Silicone column (2.65 microns × 0.53 mm × 10 m). T^a injector: 280 °C. T^a Column: 180–230 °C (8 °C/min), hold 6 min, and then 230–260 °C (10 °C/min), hold 10 min. Detector: FID (280 °C). Carrier gas flow (ml/min): N_2, 3.5 and H_2, 35. IV: 1 μl with splitting ratio of 1/25	*R*, 0.9996; 0.9984; and 0.9996. Range, 4.0–20; 0.15–0.75; and 0.080–0.4 g/l. Recovery (%), 99.62; 96.46; and 98.55. R.S.D. (%) ($n = 5$), 0.40; 1.32; and 0.65	[7]
Phenylpropanolamine, Gliceryl guaiacolate, Chlorpheniramine, Dextromethorphan	Cough-cold preparation	GC	Column: Chromosorb W (HP) (2.44 m, 0.125 in.). T^a injector: 270 °C. T^a column: 180 °C. Detector: FID (270 °C). Gas Flow (ml/min): H: 30, He: 30 y Air: 300	TA: 30 min. R.S.D. (%), 1.99; 2.55; 2.51 y 2.74	[4]
Chlorpheniramine Maleate, Phenylpropanolamine HCl	Cold tablet preparation	GLC	Column: Gas Chrom Q (1.8 m × 4 mm glass). T^a injector: 250 °C. T^a Column: 230 °C. Detector: FID (H-Air) (250 °C). Carrier gas flow (ml/min): He, 20	R.S.D. (%): 1.2 y 0.7	[2]
Salicylamide, Phenylpropanolamine HCl, Caffeine, Chlorpheniramine Maleate, Phenylephrine HCl, Pyrilamine Maleate	Capsule preparation	GLC	Column: Chromosorb W-HP (1.8 m × 2 mm glass-coil). T^a injector: 250 °C. T^a Column: 170–270 °C (9 °C/min). Detector: FID (H-Aire) (290 °C). Carrier gas flow (ml/min): N_2, 15	TA: 15 min. R.S.D. (%): 1.12–1.88	[3]
Acetaminophen, Chlorpheniramine maleate and other 12 basic drugs	Commercial preparations (cold medicine)	EKC (employing bile salts)	Fused-silica capillary (650 mm × 50 μm I.D). Buffer: 0.02 M NaH_2PO_4–0.02 M $NaBO_4$ (pH 9) + 0.1 M SDS Voltage: 20 KV. λ: 210 or 220 nm. T^a capillary: 20 °C	Concentration: 1 mg/ml. R.S.D. (%): 0.8 and 2.2. TA: <25 min	[13]

Table 1 (*Continued*)

Compounds	Sample	Technique	Conditions	Notes	Reference
Phenylpropanolamine Dextromethorphan, Chlorpheniramine maleate, Paracetamol	Cold medicine	CZE and MEC	Fused-silica capillary (600 mm × 75 μm I.D). Buffer:10 mM NaH_2PO_4-$NaBO_4$ + 50 mM SDS and 5% methanol, pH 9.0. Voltage: 25 KV. IT: 10 cm for 10 s. λ: 214 nm	Resolution: 1.2. Total migration time: 11.38 min.	[11]
Chlorpheniramine, Paracetamol Phenylpropanolamine Dextromethorphan	Common cold medicine	MECC	20 Mmol/l H_3BO_3–20 mmol/l NaOH (pH 10) with 50 mmol/l sodium deoxycholate	TA: 9 min. Recovery (%):7.86–101.5. R.S.D. (%): 0.53–2.36	[12]
Phenylpropanolamine HCl, Acetaminophen	Commercial formulation	Raman spectroscopy	Line: 514.5 nm		[1]

TA, Time of analysis; RT, Retention time; R.S.D., Relative standard deviation; LOQ, Limit of quantification; LOD, Limit of detection; MP, Mobile phase; *N*, Number of theorical plates of the column; *K*, Retention factor; ASF, Asymmetry factor of the peak.

2.2. Chemicals

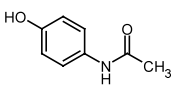
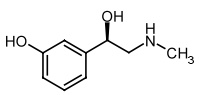
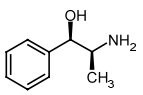
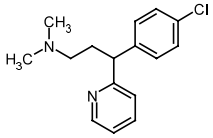
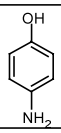
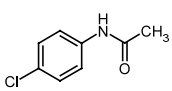
Standards of actives and impurities as well as capsules, sachets and excipients of the specialties were kindly provided by CINFA, S.A. (Pamplona, Spain). KH_2PO_4 was from Sigma (Madrid, Spain), KOH from Panreac (Barcelona, Spain), and acetonitrile and methanol from Merck (Darmstadt, Germany).

2.3. Standard solutions and sample preparation for quantitation

A stock solution of phenylephrine was prepared with 96.1 mg of phenylephrine hydrochloride exactly weighed and dissolved with methanol in a 50 ml volumetric flask. For chlorpheniramine maleate 76.9 mg were made up 100 ml with methanol. For the reference stock standard, 96 mg of acetaminophen for capsules or 122 mg for sachets were weighed in a 50 ml volumetric flask and 1 ml of phenylephrine and chlorpheniramine solutions were added. The mixture was made up the corresponding volume with methanol and treated with magnetic stirring for 10 min. 3, 4 and 5 ml of this solution were diluted in a 25 ml volumetric flask with methanol. It corresponded to 75, 100 and 125% of the nominal content in samples. They were injected three times and the peak area of each was plotted versus concentration and calibration curves were constructed using a least-square regression equation to interpolate the area of samples.

For capsules, 93.8 mg of sample coming from 20 capsules homogenate were dissolved in a 250 ml volumetric flask with methanol. After 10 min of magnetic stirring an aliquot was filtered with a 0.45 μm syringe filtration disk to the vials for injection in the HPLC system.

Table 2
Chemical structures of the assayed compounds

	MOLECULAR FORM	ESTRUCTURAL FORM	MOLECULAR WEIGHT	pKa	REF.
Acetaminophen	$C_8H_9NO_2$		151.17	9.5	[31]
Phenylephrine	$C_9H_{13}NO_2$		167.21	8.9 and 10.1	[32]
Phenylpropanolamine	$C_9H_{13}NO$		151.21	9.44 ± 0.04	[33,34]
Chlorpheniramine	$C_{16}H_{19}ClN_2$		274.80	9.1	[33,34]
4-Aminophenol	C_6H_7NO		109.13	10.46	[35]
Chloracetanilide	C_8H_8ClNO		169.61		

For sachets, 3.330 g of homogenate coming from ten sachets was suspended in a 250 ml volumetric flask with methanol/water 75:25 (v/v). The mixture was sonicated for 10 min. Ten milliliters of the suspension were diluted in a 25 ml flask with methanol and after shaken the sample was filtered with a 0.45 μm syringe filtration disk to the vials for injection in the HPLC system.

In all cases three replicates were processed.

2.4. Validation

The selectivity refers to the extent to which a method can determine particular analytes in mixtures or matrices without interferences from other components. In this assay, it was tested by running solutions containing the placebo of the specialties in the same quantities and conditions that in samples to show that there is no peak in the retention times corresponding to the analytes. Moreover, solutions of the standards with the identified impurities added were also run to show both the resolution and selectivity of the method.

The linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration. For main component assay methods, this study is generally performed by preparing standard solutions at five concentration levels, from 50 to 150% of the target analyte concentration. In this case, for capsules, acetaminophen concentrations were from 0.154 to 0.461 mg/ml, phenylephrine hydrochloride from 3.04 to 9.13 μg/ml and chlorpheniramine maleate from 1.30 to 3.89 μg/ml. For sachets the range for acetaminophen was from 0.195 to 0.583 mg/ml, because there is a slight difference in the sample content. The other two actives were in the same range. They were prepared in 25 ml volumetric flasks with 2, 3, 4, 5 and 6 ml of the stock reference solution plus methanol to complete the volume. Each point was analyzed three times. For sample linearity five solutions were identically prepared, but with the proportion of the excipients of the specialty added to each flask. For capsules it was 18 mg. For sachets, 1539.6 mg of excipients were weighed in a 25 ml flask and diluted with methanol and 2 ml of this suspension, after shaken, were added to the 25 ml volumetric flask containing the standards.

The accuracy of a method is the closeness of the measured value to the true value for the sample. For pharmaceutical studies, the most widely used approach is the recovery study, which is performed by spiking analyte in blank matrices. It was tested in the same linearity assay for the three main components. The percent recovery and R.S.D.'s were then calculated.

The precision of an analytical method is the amount of scatter in the results obtained from multiple analyses of an homogeneous sample. The first type is repeatability or intra-assay precision. Intra-assay precision data were obtained by repeatedly analyzing, in one laboratory on 1 day, six aliquots of a homogeneous sample, each of which was independently prepared according to the method procedure. The second type is intermediate precision. These data were obtained by repeating the intra-assay experiment on a different day with newly prepared mobile phase and samples.

The detection limit of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically, three times the noise level. The detection limit needs to be determined only for impurity methods in which chromatographic peaks near the detection limit will be observed.

The quantitation limit is the lowest level of analyte that can be accurately and precisely measured. Limits of detection were calculated following IUPAC recommendations $[(a + 3S_B)/b]$ for chromatographic methods 24 by extrapolating to zero concentration the standards deviation of the last three points of linearity and interpolating this value in the corresponding equation.

3. Results and discussion

During the optimization of the method two columns (X-Terra RP18 3.5 μm 100 × 4.6 mm and Symmetry RP8 5 μm 250 × 4.6 mm), three pH values (3.0, 6.0 and 7.0) with and without heptanesulphonate as ion pairing, and two organic solvents (methanol and acetonitrile) were tested. Acetaminophen was separated of the others when the mobile phase contained over 95% of aqueous

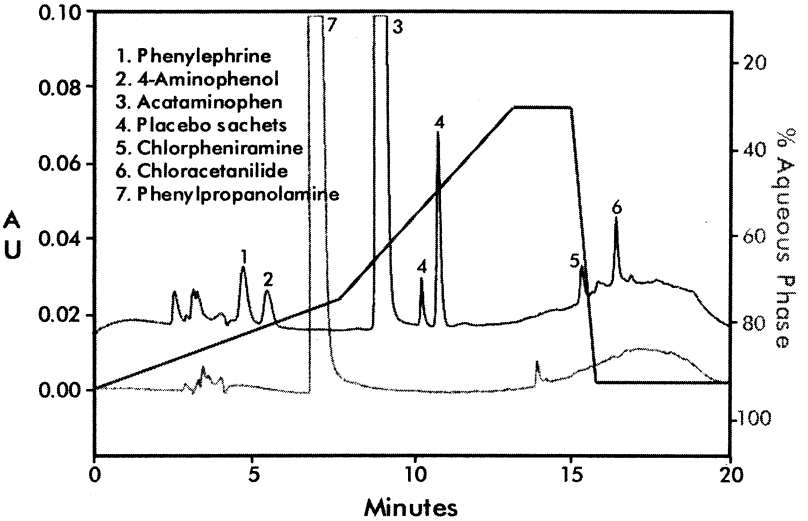


Fig. 1. Chromatogram of a mixture of phenylephrine, 4-aminophenol, acetaminophen, chlorpheniramine, chloracetanilide, and placebo. Below, phenylpropanolamine. Chromatographic conditions: Column: Symmetry RP8 5 μm (250 × 4.6 mm), T^a: 35 °C, λ: 215 nm, Mobile phase: Gradient elution with phosphate buffer 40 mM at pH 6.0 and acetonitrile, Flow-rate: 1 ml/min.

phase, but the critical point was phenylephrine and 4-aminophenol separation. It was only reached when the buffer concentration was increased from 10 to 40 mM. For ionizable compounds, an increase in ionic strength can suppress solute and silica ionization, as well as secondary interactions between them.

We could also observe that at pH 3.0 heptanosulphonate was needed as ion pairing and in such conditions all the compounds could be separated but phenylephrine and paracetamol. For phenylephrine pH 6.0 was a compromise with the lower ionization degree for both the phenol and the amino groups and in general terms it is peak shapes were better at this pH than at pH 3.0. In relation with the organic solvent, acetonitrile provided better baseline at 210 nm. This low wavelength was necessary to get enough sensitivity for the two compounds in smaller proportion (phenylephrine and chlorpheniramine). Therefore, the final chromatographic conditions were those quoted above in the previous section.

As it could be observed in Fig. 1, there is no peak in the placebos of both specialties corresponding with the migration times of the analytes. On the other hand, the known impurities of acetaminophen, 4-aminophenol and 4-chloroacetanilide, which were run together with the standards, showed both the resolution and selectivity of the method. Moreover, other actives sometimes included in this type of preparations, such as phenylpropanolamine, were also run. The selectivity of the method to these compounds could permit to have only one method for different formulations.

Main validation parameters are shown in Tables 3 and 4. Both standards and samples showed a good linearity for the three analytes in the two formulations with correlation coefficients over 0.999 except for chlorpheniramine in capsules which were over 0.99. A small bias was found in some of the regression lines, because the intercepts with their limits of confidence did not include the zero value. It could be mostly justified by the good fit of the points to the regression lines, which makes the limits of confidence for the intercept very narrow. Anyway, a calibration with three points was established in the method to avoid errors. R.S.D. values in the *intra*-assay instrumental precision ranged from 0.10 to 1.60% in capsules for the three actives and from 0.09 to 2.13% in sachets. For intermediate instrumental precision R.S.D.s ranged from 0.31 to 3.98% in capsules and from 0.19 to 1.78% in sachets, corre-

sponding again the lower value to acetaminophen and the higher value chlorpheniramine. For the *intra*-assay precision of the method R.S.D.s ranged from 0.51 to 4.27% in capsules and from 0.43 to 1.27% in sachets. For intermediate precision of the method R.S.D.s ranged from 1.29 to 3.60% for capsules and from 0.70 to 2.26% for sachets. In all cases, as it could be expected, acetaminophen, the compound with higher concentration in the formulation presented the lower variability, while, chlorpheniramine, the compound with lower content, presented the higher variability. With these values and the intervals of acceptance (95–105% for acetaminophen and 90–110% for phenylephrine and chlorpheniramine) three replicates of each sample ought to be measured for quantification.

Recoveries do not statistically differ from 100% (t-test, $P < 0.05$) in any case and R.S.D. for recoveries range from 0.44 to 5.31% in capsules and from 0.32 to 4.92% in sachets, being again the higher values for chlorpheniramine which is under 1% with respect to acetaminophen. Limits of detection are not necessary for acetaminophen. They were 1.2×10^{-4} mg/ml for phenylephrine and 1.5×10^{-4} mg/ml for chlorpheniramine, which are under the necessary values for the method.

Although a formal robustness assay has not been achieved, this method has been applied over 6 months in two pharmaceutical formulations (capsules and sachets) and it has always passed the system suitability test.

4. Conclusion

A HPLC method has been developed for acetaminophen, phenylephrine and chlorpheniramine

Table 3
Main validation parameters of capsules

			Capsules		
			Acetaminophen	Fenylephrine Hydrochloride	Chlorpheniramine Maleate
Standards linearity		Intercept	-0.6 ± 0.7	-0.05 ± 0.06	-0.12 ± 0.06
		Slope	301 ± 2	667 ± 9	563 ± 22
		r	0.9999	0.9998	0.998
		Range (mg/ml)	0.15376–0.46128	0.00304–0.00913	0.00130–0.00389
Sample linearity		Intercept	-0.4 ± 0.7	-0.01 ± 0.04	-0.11 ± 0.08
		Slope	307 ± 2	672 ± 6	564 ± 29
		r	0.9999	0.9999	0.996
Accuracy % Recovery		Standard	100.0 ± 0.3	100.0 ± 0.6	100 ± 1
		R.S.D. (%)	0.56	1.10	2.26
	Sample		100.0 ± 0.2	101.6 ± 0.4	99 ± 3
	R.S.D. (%)		0.44	0.70	5.31
Standards precision instrumental	Intra-assay ($n = 6$)	Mean (mg/ml)	0.3075 ± 0.0003	0.00609 ± 0.00009	0.00260 ± 0.00003
		R.S.D. (%)	0.10	1.48	1.08
	Intermediate ($n = 12$)	Mean (mg/ml)	0.3078 ± 0.0006	0.00609 ± 0.00005	0.00260 ± 0.00002
		R.S.D. (%)	0.31	1.25	0.94
Sample precision instrumental	Intra-assay ($n = 6$)	Mean (mg/ml)	0.3107 ± 0.0004	0.00625 ± 0.00004	0.00250 ± 0.00004
		R.S.D. (%)	0.11	0.59	1.60
	Intermediate ($n = 12$)	Mean (mg/ml)	0.309 ± 0.001	0.00616 ± 0.00007	0.00245 ± 0.00006
		R.S.D. (%)	0.76	1.78	3.98
Precision methods	Intra-assay ($n = 6$)	Mean (mg per cap)	503 ± 3	9.9 ± 0.1	4.0 ± 0.2
		R.S.D. (%)	0.51	1.30	4.27
	Intermediate ($n = 12$)	Mean (mg per cap)	498 ± 4	10.0 ± 0.1	3.97 ± 0.09
		R.S.D. (%)	1.29	1.87	3.60

Table 4
Main validation parameters of sachets

			Sachets		
			Acetaminophen	Fenylephrine Hydrochloride	Chlorpheniramine Maleate
Standards Linearity		Intercept	-0.3 ± 0.6	-0.06 ± 0.06	-0.22 ± 0.04
		Slope	310 ± 2	694 ± 9	621 ± 13
		r	0.99997	0.9998	0.9994
		Range (mg/ml)	0.19440–0.58320	0.00304–0.00913	0.00130–0.00389
Sample Linearity		Intercept	-0.6 ± 0.6	-0.02 ± 0.05	-0.2 ± 0.1
		Slope	310 ± 2	680 ± 8	609 ± 38
		r	0.99997	0.9998	0.995
Accuracy % Recovery		Standard	100.0 ± 0.2	100.0 ± 0.5	100 ± 1
		R.S.D. (%)	0.32	0.91	1.79
	Sample		99.9 ± 0.2	99.0 ± 0.6	98 ± 3
	R.S.D. (%)		0.33	1.14	4.92
Standards precision instrumental	Intra-assay ($n = 6$)	Mean (mg/ml)	0.3888 ± 0.0004	0.00609 ± 0.00006	0.00260 ± 0.00006
		R.S.D. (%)	0.09	0.97	2.13
	Intermediate ($n = 12$)	Mean (mg/ml)	0.3888 ± 0.0005	0.00609 ± 0.00006	0.00260 ± 0.00003
		R.S.D. (%)	0.19	1.53	1.59
Sample precision instrumental	Intra-assay ($n = 6$)	Mean (mg/ml)	0.3882 ± 0.0008	0.00603 ± 0.00003	0.00243 ± 0.00002
		R.S.D. (%)	0.19	0.40	0.97
	Intermediate ($n = 12$)	Mean (mg/ml)	0.3884 ± 0.0005	0.00607 ± 0.00004	0.00242 ± 0.00003
		R.S.D. (%)	0.19	0.97	1.78
Precision methods	Intra-assay ($n = 6$)	Mean (per mg)	0.493 ± 0.002	0.0094 ± 0.0001	0.00546 ± 0.00007
		R.S.D. (%)	0.43	1.09	1.27
	Intermediate ($n = 12$)	Mean (per mg)	0.491 ± 0.002	0.0093 ± 0.0001	0.00544 ± 0.00004
		R.S.D. (%)	0.70	2.26	1.15

and related impurities measurement in capsules and tablets as pharmaceutical form with gradient elution in a single run. The method described in theis study was found suitable to determine concentrations in the range 0.15 to 0.46 mg/ml for acetaminophen, 0.003 to 0.009 mg/ml for phenylephine and from 0.001 to 0.004 mg/ml for chlorpheniramine, precisely and accurately, in agreement with the validation parameters obtained. Limits of detection for the two actives with lower concentration were 1.2×10^{-4} mg/ml for phenylephrine and 1.5×10^{-4} mg/ml for chlorpheniramine, values which are under the lowest expected concentrations in the samples.

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