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Development and validation of a liquid chromatographic method for the determination of related substances in verapamil hydrochloride

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Abstract

The development of a reversed-phase liquid chromatographic method for the determination of related substances in verapamil hydrochloride is described. The method is based on the use of a simple mobile phase on a specialty base-deactivated reversed-phase column. It enables the resolution of 13 related compounds from the parent drug and from each other. Validation of the method showed it to be reproducible, selective, accurate and linear over the concentration range of analysis with a limit of detection of 0.5 μg ml^{-1}. The developed method proved to be a real improvement compared with the LC test for chromatographic purity described in the USP monograph for verapamil hydrochloride. © 1997 Elsevier Science B.V.

Keywords: Verapamil hydrochloride; Related substances; Reversed-phase liquid chromatography

1. Introduction

Verapamil hydrochloride, (benzeneacetonitrile, α-[3-[[2-(3,4-dimethoxyphenyl)-ethyl]-methyl-amino] propyl]-3,4-dimethoxy-α-(1-methylethyl)-, monohydrochloride, (±)-) is a calcium channel blocker belonging to the phenylalkylamine class [1,2]. It is an important therapeutic agent for the management of angina and atrial tachyarrhythmias [3,4].

The related impurities that are likely to be present in finished verapamil hydrochloride are reported in Fig. 1.

Several HPLC methods for the assay of verapamil hydrochloride in dosage forms and biological fluids have been described but no data on the related impurities separation are reported [5–8]. The USP XXIII monographs for verapamil hydrochloride tablets and injection [9] provide an HPLC method for the determination of the chromatographic purity based on the use of a conventional reversed-phase column of ODS-silica and a mobile phase consisting of an acetonitrile-acetate buffer mixture to which a competing amine is added. This method, previously described by Lacroix et al. [10], was tried on the 13 selected impurities. Though it enables the resolution of

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verapamil from all available impurities, most of them are eluted not well resolved in the first portion of the chromatogram and impurities I and VI co-elute. A recent proposal for a change of the USP monograph [11] nearly conforms with the former method. In fact, the main modification consists in a decrease of the acetonitrile content in the mobile phase causing the retention times to increase without any substantial improvement to the impurities chromatographic separation. Impurities I, II and III co-elute near the solvent front and impurity XIII is not eluted from the column.

The method described in the European Pharmacopoeia monograph for the control of related substances in verapamil hydrochloride is a thin-layer chromatographic method where the test substance is compared with the European Pharmacopoeia reference standard [12]. The TLC method does not resolve all the available related compounds from each other.

None of the above-mentioned methods allows unresolved compounds to be determined individually to test for compliance with the specifications set in the monographs.

This paper describes the development and validation of a chromatographic method based on the use of a simple mobile phase on a specialty base-deactivated reversed phase column. It allows the separation of all the selected impurities from verapamil and from each other without the addition of a competing base to the mobile phase.

2. Experimental

2.1. Samples

Ten samples of verapamil hydrochloride of four different origins were analysed (Recordati, Milano, Italy; Alfa Chemicals Italiana, Milano, Italy; Knoll AG, Ludwigshafen, Germany and Orion Corp, Espoo, Finland).

The available potential impurities (precursors, by products and degradation products) were: *N,N'*-methyl-*N,N'*-(3,4-dimethoxyphenethyl)-1,3-propanediamine (I); 3,4-dimethoxy-*N*-methyl benzeneethanamine hydrochloride (II); 3,4-dimethoxy-*N*-dimethyl benzeneethanamine hydrochloride (III); 3,4-dimethoxy-*N*-(3-chloropropyl)-*N*-methyl benzeneethanamine hydrochloride (IV); 3,4-dimethoxybenzylalcohol (V); benzeneacetonitrile, 3,4-dimethoxy-α-[3-(methylamino)-propyl]-α-(1-methylethyl) hydrochloride (VI, USP related compound A); 3,4-dimethoxybenzaldehyde (VII); benzeneacetonitrile, α-[3-[[2-(3,4-dimethoxyphenyl)-ethyl]-methylamino] propyl] -3,4-dimethoxy-α-ethyl hydrochloride (VIII); benzeneacetonitrile, α-[2-[[2-(3,4-dimethoxyphenyl)-ethyl]-methylamino] ethyl] -3,4-dimethoxy-α-(1-methylethyl) hydrochloride (IX, USP related compound B); *N*-norverapamil hydrochloride (X); benzeneacetonitrile, 3,4-dimethoxy-α-(1-methylethyl) (XI); 1-(3,4-dimethoxyphenyl)-2-methyl-1-propanone (XII); *N,N*-bis[4-(3,4-dimethoxyphenyl)-4-cyano-5-methylhexyl]-*N*-[2-(3,4-dimethoxyphenyl)-ethyl] amine hydrochloride (XIII) (Fig. 1).

Impurities IV, V, VI, VII, IX, X and XI were kindly provided by Recordati; impurity VIII was from Alfa Chemicals Italiana; impurities I, II, III and XII were from Knoll AG; impurity XIII was from Orion Corp.

The mass, IR and NMR spectra of the mentioned compounds were in compliance with their respective structures.

2.2. Chemicals

HPLC-grade acetonitrile was supplied by Merck (Darmstadt, Germany). Water was bidistilled.

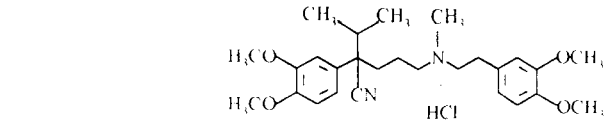
Potassium phosphate monobasic and potassium phosphate dibasic were from Farmitalia Carlo Erba (Milan, Italy).

All the other reagents were of analytical grade.

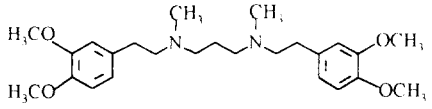
2.3. HPLC apparatus and chromatographic conditions

HPLC analyses were performed using a high pressure quaternary pump (HP 1050) equipped with an HP 1050 autosampler, an HP 1040M diode array detector and a MS-DOS 3D workstation, all from Hewlett-Packard (Avondale, USA).

The chromatografic column was a 5-μm Suplex pKb-100, 250 × 4.6 mm I.D. (Supelco, Bellefonte, PA USA).



Verapamil hydrochloride



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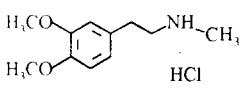

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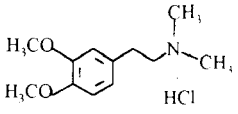

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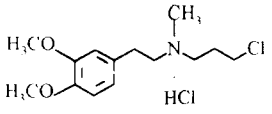

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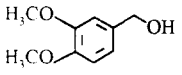

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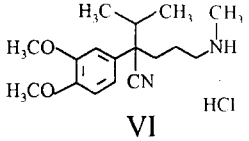

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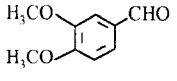

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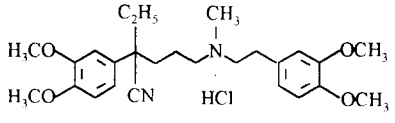

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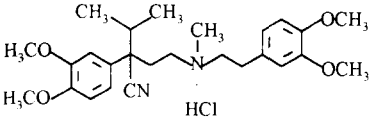

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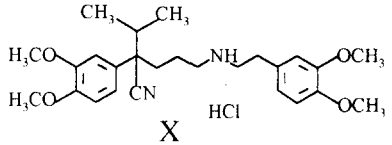

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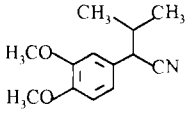

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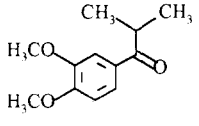

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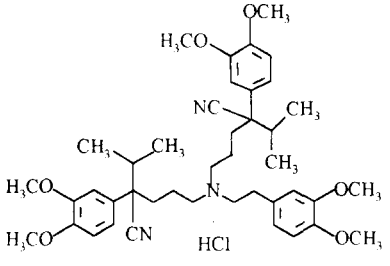

XIII

Fig. 1. Chemical structures of verapamil and related compounds

The eluents were:
(A) Potassium phosphate dibasic (pH 7.20; 0.04 M).
(B) Acetonitrile.

A double isocratic elution was performed as described in Table 1. The analyses were performed at room temperature at a flow rate of 1.5 ml min^{-1}. The monitoring wavelength was 278 nm and the injection volume was 10 μl.

2.4. Preparation of the solutions and procedure

All the solutions were prepared in 0.04 M potassium phosphate monobasic adjusted to pH 3.0 with phosphoric acid (solution A). A low pH value was chosen to assure a greater stability of all the available compounds during the method development.

2.4.1. Impurity stock solution

Quantities between 1.3 and 1.6 mg of verapamil hydrochloride and impurities were dissolved together in the solution A and diluted to 100 ml with the same solution. Injections of this mixture were made to evaluate the capacity of the method in separating all the potential impurities one from each other and from verapamil. Moreover, spectral information for each analyte was obtained for the determination of their respective response factors.

2.4.2. Sample solution

Verapamil hydrochloride (25 mg) was accurately weighed and dissolved in 5 ml of the solution A (5 mg ml^{-1}).

Table 1
HPLC double isocratic programme

Time (min)	Eluent A[a]	Eluent B[b]	Description
0–22	63	37	First isocratic step
22–27	63 → 50	37 → 50	Linear gradient
27–35	50	50	Second isocratic step
35–36	50 → 63	50 → 37	Switch to initial mobile phase
36–50	63	37	Equilibration

[a]Eluent A: potassium phosphate dibasic (pH 7.20; 0.04M).
[b]Eluent B: acetonitrile.

2.4.3. Reference solution

A portion of the sample solution (0.3 ml) was diluted to 100 ml with the solution A to give a concentration of 0.015 mg ml^{-1}.

2.4.4. Resolution solution

Quantities between 4.9 and 5.1 mg of verapamil hydrochloride and impurity IX (USP reference compound B) were dissolved together in the solution A and diluted to 20 ml in the same solution. A portion of this solution (1 ml) was then further diluted to 10 ml with the solution A (0.025 mg ml^{-1} of each analyte). Resolution between verapamil and impurity IX was calculated from replicate injections ($n = 6$) according to the method described in the European Pharmacopoeia [13]. The symmetry factor for verapamil peak was also calculated.

2.4.5. Spiked sample solutions

Five sample solutions were spiked with each impurity in different amounts (0.02, 0.05, 0.15, 0.30 and 0.50%) to evaluate the accuracy and precision of the method. The reference solution was separately injected and the average recovery and R.S.D. were calculated taking account of the response factors.

2.4.6. Calibration solutions

Ten calibration solutions, each containing verapamil hydrochloride and all the impurities, were prepared to check the response linearity. Their concentrations were equivalent to an impurity content of 0.01, 0.02, 0.03, 0.05, 0.10, 0.15, 0.20, 0.30, 0.50 and 1.00% with respect to verapamil in the sample solution. Three replicate injections of each solution were made and from the obtained peak areas the correlation curve for each compound with respect to its concentration was determined. The equation lines $y = mx + b$ were established and the correlation coefficients were calculated.

2.4.7. LOD and LOQ determination

Replicate injections ($n = 6$) of the solution A were made. The analysis time for each injection was 15 min. The average noise level and its standard deviation were calculated in absorbance

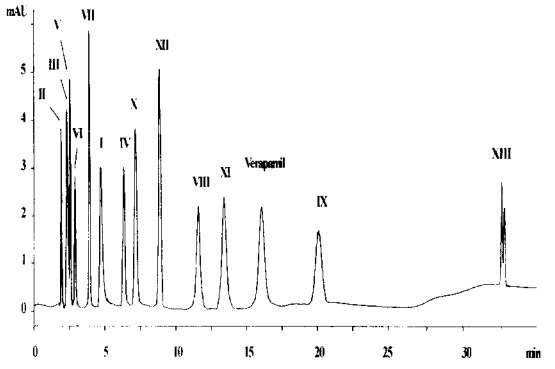

Fig. 2. Chromatographic separation of verapamil and related substances obtained by the proposed method (impurity stock solution).

units. The average noise level was multiplied by factors of 2 and 4 to give proposed absorbance limits of detection (LOD) and quantitation (LOQ), respectively. These limits were then converted into their equivalent concentration values using the peak area of a known concentration of verapamil hydrochloride as standard. A solution containing verapamil hydrochloride and all the impurities at their detection limit concentrations (0.01%) was prepared. Peak areas were recorded for each analyte and the respective standard deviations were calculated. The detection and quantitation limits were then verified for each impurity taking into account their respective response factors.

3. Results and discussion

Fig. 2 shows the resolution of 13 potential impurities from verapamil. All the related compounds were resolved from the drug and from each other. Impurity XIII was eluted as a doublet during the second isocratic step. The comparison between the proposed procedure and the USP modified method [11] is reported in Table 2.

At the detection wavelength a good uniformity of response was observed for all except two substances. At 278 nm, in fact, impurities VII and XII have a response factor about five and three times greater than that for verapamil, respectively. All other substances give response factors between 73 and 119% of the value for verapamil and could be considered sufficiently similar for the purpose of the limit test for the drug substance (Table 4).

3.1. Precision, accuracy, sensitivity and linearity

The variation in retention time among six replicate injections of verapamil reference solution was very little, giving a relative standard deviation (R.S.D.) of 1.7%

Recoveries for all the analytes, determined in quadruplicate on the spiked sample solutions in comparison with the reference solution, ranged from about 86–109%. Peak-area repeatabilities were less than 1.5% for 0.5 and 0.3% contents, less than 5% for 0.15 and 0.05% contents and less than 13% for 0.02% content. (Table 3).

The LOD and LOQ were about 0.5 μg ml^{-1} (0.01%) and 1.0 μg ml^{-1} (0.02%), respectively, for all the analytes except impurities VII and XII whose LOD and LOQ results were lower owing to their response factor. Linearity was satisfactory for all the analytes, giving correlation coefficients ranging from 0.9952 to 0.9999. LOD and LOQ for each compound are reported in Table 4 along with linearity data.

Table 2
Comparison between the proposed method and the USP modified method

Compound	RRT[a]	RRT[b]
II	0.14	0.18
III	0.16	0.18
V	0.18	0.30
VI	0.19	0.31
VII	0.28	0.60
I	0.31	0.17
IV	0.39	0.23
X	0.44	0.91
XII	0.57	2.18
VIII	0.75	0.68
XI	0.88	3.49
Verapamil	1.00	1.00
IX	1.34	0.84
XIII	1.98	Not eluted

[a]Relative retention times by the proposed method (verapamil r.t. about 16 min).
[b]Relative retention times by the USP modified method (verapamil r.t about 8 min).

Table 3
Accuracy and precision data for verapamil related compounds calculated on spiked sample solutions

Compound	Spiked amount (%)									
	0.02		0.05		0.15		0.30		0.50	
	Recovery (%)	R.S.D. ($n=4$)	Recovery (%)	R.S.D. ($n=4$)	Recovery (%)	R.S.D. ($n=4$)	Recovery (%)	R.S.D. ($n=4$)	Recovery (%)	R.S.D. ($n=4$)
I	99.3	8.2	102.4	3.8	101.3	1.9	97.9	1.1	105.4	0.7
II	104.9	7.8	106.9	4.1	99.7	1.8	102.3	0.8	103.8	1.0
III	102.4	9.5	99.8	2.9	101.1	0.9	99.4	0.4	101.0	0.3
IV	109.2	6.2	108.8	4.6	86.9	2.3	101.0	1.2	106.2	0.8
V	87.1	6.0	106.0	2.1	86.7	1.0	92.7	0.3	108.9	0.2
VI	108.8	12.8	109.2	1.0	109.0	1.0	105.2	0.4	103.2	0.3
VII	105.1	5.8	108.7	0.2	94.7	0.3	102.7	0.8	109.1	0.2
VIII	104.7	7.7	101.0	4.7	99.3	3.3	103.4	1.2	100.1	1.2
IX	86.5	9.7	106.2	4.8	98.7	3.1	100.7	1.5	109.2	0.3
X	105.3	6.4	108.2	4.8	102.7	1.5	88.7	0.9	100.6	0.6
XI	109.0	11.7	109.4	5.0	102.2	3.5	104.1	1.2	109.3	1.3
XII	99.8	10.6	92.6	3.9	100.2	3.0	98.8	1.3	102.7	1.2
XIII	89.6	12.3	99.7	4.9	97.8	3.1	96.9	1.2	100.2	1.3

3.2. Stability of solutions

During the initial stage of the method development the impurity stock solution was prepared by dissolving all the analytes in the eluent A (pH 7.20). The appearance of some additional peaks in the chromatogram was observed in the course of 24 h at room temperature. As the optimum pH range of stability for verapamil hydrochloride solutions was shown to be about 3.2–5.6 [6], the stock solution was prepared again using a buffer at a lower pH (solution A, pH 3). No additional peaks appeared in the chromatogram after a period of 24 h at room temperature.

3.3. Ruggedness

During the method development it became clear that the preparation of the mobile phase was critical for ensuring the separation of all analytes. Particularly, a slight decrease in the pH value of the phosphate buffer caused the impurities VII and I to co-elute, whereas a slight increase caused the co-elution of impurities VIII and XI. Moreover, slight variations in retention times were observed using mobile phases prepared on different days by different analysts and tested on different instruments. On no occasion, however, did this affect the separation of the principal peak from all the impurities. Since the separation of verapamil potential impurities is sensitive to slight variations in the pH value, a performance requirement has to be included in the method for realizing whether or not complete separation of all analytes is likely to be achieved. As reference standards of all potential impurities are not readly available, the resolution requirement between verapamil and impurity IX was considered satisfactory. The resolution requirement was evaluated to be 5 or more to achieve a complete separation of all the analytes. The symmetry factor for the verapamil peak was 0.93.

The column-to-column reproducibility was evaluated injecting the impurity stock solution on three columns from the same manufacturer and containing the same brand of packing material. The elution order and the resolution of the compounds were not affected and only slight variations in retention times were observed.

Table 4
Response and linearity data for verapamil and related impurities

Compound	RRF[a]	LOD (%)[b]	LOQ (%)[b]	R[c]
Verapamil	1.00	0.008	0.016	0.9993
I	0.87	0.010	0.020	0.9963
II	1.04	0.008	0.016	0.9992
III	0.95	0.008	0.016	0.9989
IV	0.73	0.012	0.024	0.9964
V	1.19	0.008	0.016	0.9952
VI	0.78	0.010	0.020	0.9996
VII	5.55	0.002	0.004	0.9971
VIII	1.03	0.008	0.016	0.9991
IX	0.97	0.012	0.024	0.9996
X	1.04	0.008	0.016	0.9999
XI	1.02	0.010	0.020	0.9974
XII	3.57	0.004	0.008	0.9993
XIII	1.18	0.008	0.016	0.9979

[a]Response factors relative to verapamil determined on the impurity stock solution.
[b]LOD: limit of detection (2 × baseline noise). LOQ: limit of quantitation (4 × baseline noise). LOD and LOQ were determined on a solution containing each analyte at a level of 0.01% ($n = 3$) taking into account the respective RRF.
[c]Correlation coefficient calculated on the calibration solutions ($n = 3$).

Four replicate injections of verapamil hydrochloride sample solution were made every day over a period of 3 weeks to evaluate both the intraday and the interday repeatability. The intraday relative standard deviation ranged between 0.07 and 0.15%; the interday relative standard deviation ranged between 0.16 and 0.55%.

3.4. Analysis of verapamil hydrochloride raw materials

Impurity levels on ten samples of verapamil hydrochloride from different sources were estimated under the described conditions by comparison with verapamil reference solution. In all cases, no single impurity exceeded 0.06% and total impurities were not more than 0.2%. The samples were tested in triplicate. The results relative to the total impurity levels (Table 5) were comparable with those previously obtained by the USP XXIII modified method on the same samples.

4. Conclusions

The proposed HPLC method seems to be a real improvement in the separation of the available impurities from verapamil and from each other when compared with the USP modified method. The latter, being based on the use of a conventional reversed-phase column with the addition of a competing amine to the mobile phase, gives rise to both bad peak shape and column life shortening. Moreover, it allows the separation of all the impurities from the bulk drug but not from each other. In fact, impurities I, II and III co-elute very near the solvent front and impurity XIII is not eluted from the column. On the contrary, the method described in this paper is based on the use of a simple mobile phase on a specialty base-deactivated reversed-phase column by which competing amines are not required to obtain a good peak shape. Moreover, a substantial improvement in the impurities separation is achieved. In fact, all the available potential impurities are separated from verapamil and from each other and impurity XIII is eluted from the column during the second isocratic step. Consequently, the developed method allows all the compounds to be determined individually to test for compliance with the specifications set in the various Pharmacopoeia monographs. Furthermore, it is suitable for controlling the purity of verapamil hydrochloride from different sources.

Table 5
Total impurity levels determined on ten samples of verapamil hydrochloride from different sources

Sample no.	1	2	3	4	5	6	7	8	9	10
Impurity level (%)[a]	0.08	0.10	0.11	0.12	0.04	0.06	0.05	0.08	0.02	0.03
R.S.D. ($n = 3$)	2.3	1.8	0.6	0.9	3.5	2.6	4.1	1.2	5.3	5.8

[a]Mean impurity level determined on three replicate analyses of verapamil hydrochloride sample solutions.

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