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The HPLC determination of propane-1,2,3-triyl trinitrite and impurities: (2*RS*)-3-hydroxypropane-1,2-diyl dinitrate and 2-hydroxypropane-1,3-diyl dinitrate in ointment

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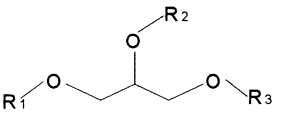
Abstract

A HPLC method for determination of propane-1,2,3-triyl trinitrate and impurities: (2*RS*)-3-hydroxypropane-1,2-diyl dinitrate and 2-hydroxypropane-1,3-diyl dinitrate ointment was developed. The conditions for good separation of constituents, while avoiding vehiculum interference were established. The results feature of high accuracy and good precision. For individual constituents R.S.D. is ranged from 0.7 to 9.9%, while recovery was 100.1% for propane-1,2,3-triyl trinitrate and 95.1–99.0% for impurities. It has been found that propane-1,2,3-triyl trinitrate used in medicine in the form of ointment contains such impurities which can be identified and quantified at relatively low concentrations of 70 ng ml^{-1}.

Keywords: Impurities; (2*RS*)-2,3-Dihydroxy-propyl nitrate; 2-Hydroxy-1-(hydroxymethyl)ethyl nitrate; (2*RS*)-3-Hydroxypropane-1,2-diyl dinitrate; 2-Hydroxypropane-1,3-diyl dinitrate; HPLC; Drug analysis

1. Introduction

Organic nitrates are still commonly used in therapy of chronic illnesses resulting from blood vessel contraction [1]. Propane-1,2,3-triyl trinitrate (glyceryl trinitrate) (A) of formula in which $R_1 = R_2 = R_3 = NO_2$ belongs to most important nitrates used for that purpose.



According to European Pharmacopoeia [2] propane-1,2,3-triyl trinitrate of formula mentioned above can be accompanied by other substances referred to as impurities in the pharmacopoeia. Such impurities of limited concentration include: $R_1 = NO_2$, $R_2 = R_3 = H$, (2*RS*)-2,3-dihydroxypropyl nitrate (B); $R_1 = R_3 = H$, $R_2 = NO_2$, 2-hydroxy-1-(hydroxymethyl)ethyl nitrate (C); $R_1 =$

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$R_2 = NO_2$, $R_3 = H$, (2*RS*)-3-hydroxypropane-1,2-diyl dinitrate (D); and $R_1 = R_3 = NO_2$, $R_2 = H$, 2-hydroxypropane-1,3-diyl dinitrate (E).

In the pharmacopoeia mentioned above the HPLC method is recommended for purity assessment by comparing the additional peak areas to that of active substance at appropriate dilution. Thus, no full identification of impurities is taken into account. Similarly, both British Pharmacopoeia 2000 [3] and American Pharmacopoeia [4] do not predict determination of impurities in ointments containing glyceryl trinitrate probably due to difficulties in analysis of such drugs.

Although various methods such as chromatography [5–7], thin-layer chromatography [8,9], spectrophotometric techniques [10,11], NMR spectroscopy [12] and electroanalytical [13,14] for determining nitrates have been presented in available literature, there are no papers dealing with concurrent identification and quantification of active substance and impurities in ointments. As glyceryl trinitrate is used in many drugs, including ointments [15], in this paper the conditions for concurrent identification and quantitative determination of active substance and impurities, if any, are established by employing the HPLC method with reversed-phase partition.

2. Experimental

2.1. Apparatus and reagents

La Chrom chromatograph manufactured by Merck-Hitachi, equipped with DAD detector and autosampler and the D-7000 HSM software package.

Column of 250 × 4 mm in size, packing: octadecyl silane bonded chemically on the surface of spherical silica of 5 μm in size (Lichrospher 100 RP-18).

AT-216 analytical balance manufactured by Mettler; accuracy 0.01 mg.

Methanol of gradient-grade for chromatographic applications, supplied by Merck.

2.2. Standards and chemicals

The standards used meet the requirements specified in Ph.E.

Standard solution: 5% ethanol solution of nitroglycerine, s.2/2001 manufactured by ZPS "Pronit" (Poland).

(2*RS*)-2,3-Dihydroxy-propyl nitrate s. 30908-47E, 2-hydroxy-1-(hydroxymethyl)ethyl nitrate s. 32-460-38E, 2-hydroxypropane-1,3-diyl dinitrate s. 29875-23H, (2*RS*)-3-hydroxypropane-1,2-diyl dinitrate s. 29700-53G (Cerilliant Corporation).

Nitrocard ointment, s. 001125 ("Chema-Elektromet", Rzeszów, Poland).

A reference material prepared under laboratory conditions, containing glyceryltrinitrate 2.00 and vehiculum: vaseline 44.00, lanoline 44.00, distilled water 10.00, butylhydroxyanisole 0.01.

2.3. Standard solutions

Solutions of individual impurities at concentrations of 1 and 2 μg ml^{-1} were prepared in the mixture of methanol and water (1:1). The standard solution of propane-1,2,3-triyl trinitrate of 0.1 mg ml^{-1} in concentration was prepared.

2.4. Sample solutions

An amount of the drug corresponding to 2.5 mg of propane-1,2,3-triyl trinitrate was weighed with the accuracy of 0.1 mg, added to 25.0 ml of the mixture of methanol and water (1:1) and heated in the water bath for 15 min at 50 °C. Then, the sample was taken out from the bath and intensively shaken for 1 min. The procedure was repeated once again. The solution was cooled, filtered and filled up to 25.0 ml with the mixture of methanol and water (1:1).

2.5. Vehiculum solution

Similarly, the placebo solution was prepared by extraction of appropriate vehiculum constituents for the ointment under examination.

2.6. Quantitative analysis

The determination was carried out at room temperature. The mixture of methanol and water (1:1) at flow rate of 0.8 ml min^{-1} was used as mobile phase. The measurements were made at wavelength $\lambda = 210$ nm. The injection volume was fixed at 30 μl. The standard and sample solutions were introduced in the same volumes into the column and corresponding chromatograms were taken. Individual peaks were identified from retention time, while concentrations were derived from the peak area for appropriate standard and sample solutions.

3. Results and discussion

To verify the suitability for determining glyceryl trinitrate and impurities, if any, the method was subjected to preliminary validation [16].

The chromatogram peaks were identified for appropriate solutions of constituents under investigation and the effect of vehiculum was examined. The following retention times were obtained: approximately 10.3 min for propane-1,2,3-triyl trinitrate and 3.05, 2.94, 4.53 and 4.13 min for impurities B, C, D and E, respectively. An example of chromatograms is presented in Figs. 1 and 2.

It is clearly visible that glyceryl trinitrate is well separated from impurities B, C, D and E under conditions mentioned above. Unfortunately, no satisfactory separation was achieved for impurities B and C, both of similar retention times. As no peaks originated from impurities B and C were found, the quantitative analysis was limited to remaining impurities D and F being also subjected to semi-quantitative analysis in European Pharmacopoeia in respect of basic substance.

Further investigations were focused on an effect of vehiculum used in the drug under examination. To do it the solutions containing ointment vehiculum at concentrations corresponding to those of the drug were prepared. Although individual signals in the form of peaks were recorded, its retention times did not comply with those of impurities D and E as well as glyceryl trinitrate, thus indicating no effect on the determination of these constituents (Fig. 3).

It was found that the chromatogram peaks of retention time below 2 min originated from the vehiculum constituents and mobile phase solvents. Such they should be ignored when assessing the drug purity.

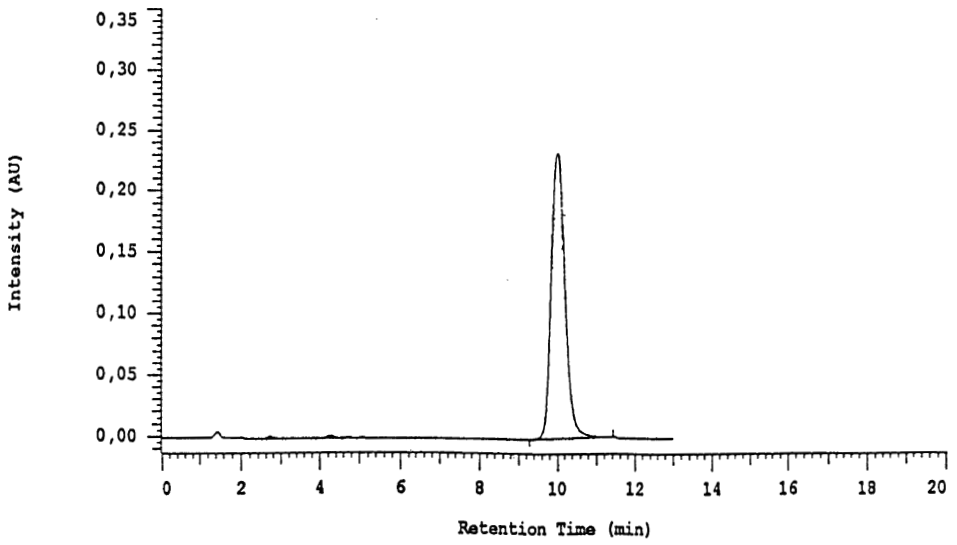

Fig. 1. Chromatogram of propane-1,2,3-triyl trinitrate standard solution.

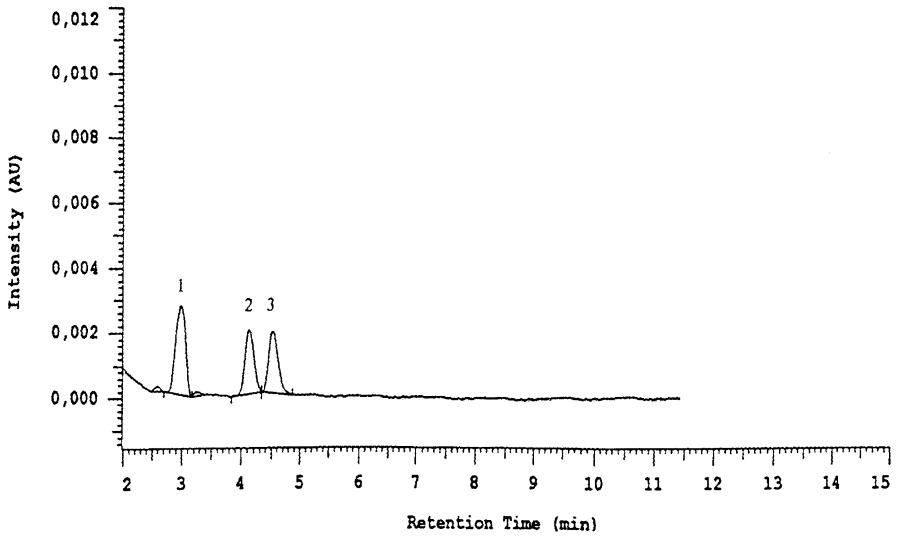

Fig. 2. Chromatogram of impurities solution. Peaks: mixture (2*RS*)-2,3-dihydroxy-propyl nitrate and 2-hydroxy-1-(hydroxymethyl)ethyl nitrate (1); 2-hydroxypropane-1,3-diyl dinitrate (2); (2*RS*)-3-hydroxypropane-1,2-diyl dinitrate (3).

The limit of detection was derived from the concentration that corresponds to the mean value obtained from blank test plus three standard deviations.

The limit of determination corresponds to the limit of detection plus three standard deviations for blank test. The results are presented below:

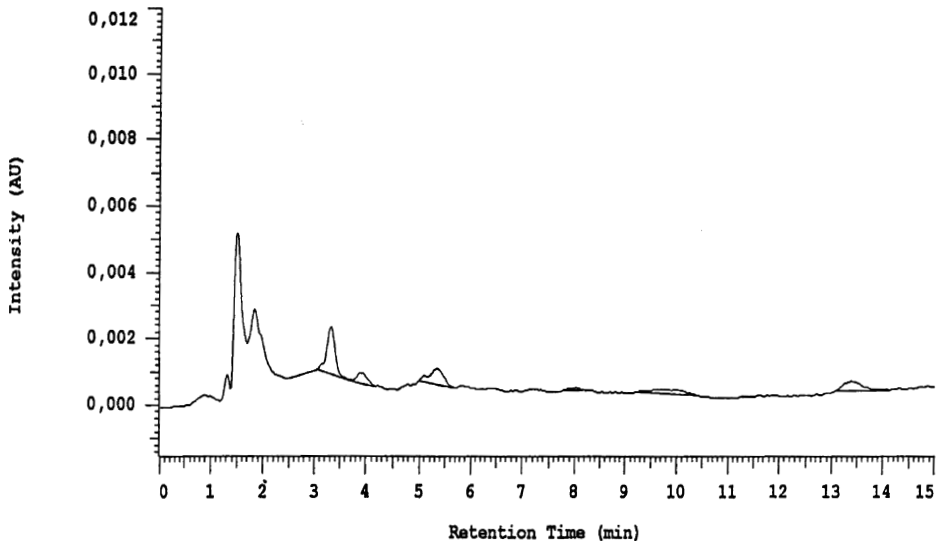

Fig. 3. Chromatogram of placebo solution. The analysis was carried as described in the text.

	Limit of detection	Limit of determination
Glyceryl trinitrate	90 ng ml^{-1}	120 ng ml^{-1}
Impurities B and C	90 ng ml^{-1}	150 ng ml^{-1}
Impurities D and E	70 ng ml^{-1}	110 ng ml^{-1}

Standard solutions of appropriate constituents at concentrations ranging from 50 to 150% of determined value were used for linearity checking. The analytical curves demonstrate linear behaviour within the range mentioned above and can be described with the following equations and determination coefficients R^2:

Glyceryl trinitrate	(from 50 to 150 μg ml^{-1})	$y = 27\,605x + 25\,301$	$R^2 = 0.9991$
Impurities B and C	(from 1 to 3.2 μg ml^{-1})	$y = 12\,932x + 1750.3$	$R^2 = 0.9864$
Impurity D	(from 0.5 to 1.6 μg ml^{-1})	$y = 13\,945x + 2328.3$	$R^2 = 0.9613$
Impurity E	(from 0.5 to 1.6 μg ml^{-1})	$y = 16\,194x + 911.2$	$R^2 = 0.9797$

A reference sample containing glyceryl trinitrate at concentration of 2.050% and impurities D (0.38%) and E (0.29%) at given concentrations recalculated into active substance were used for precision evaluation. Chromatogram obtained under the conditions described above revealed the presence of a peak assigned to the active substance—glyceryl trinitrate, alongside with the peaks of impurities D and E and additional peaks resulting from the matrix (Fig. 4). The peaks of analysed components were very well resolved and no interference was observed with the peaksassigned to placebo (Fig. 3), which can be seen on both chromatograms. This was further confirmed in quantitative analysis (Table 1) as the results obtained were comparable to the amounts of components used to prepare the model ointment.

The precision was evaluated by computing the mean value (x), coefficient of variation in percen-

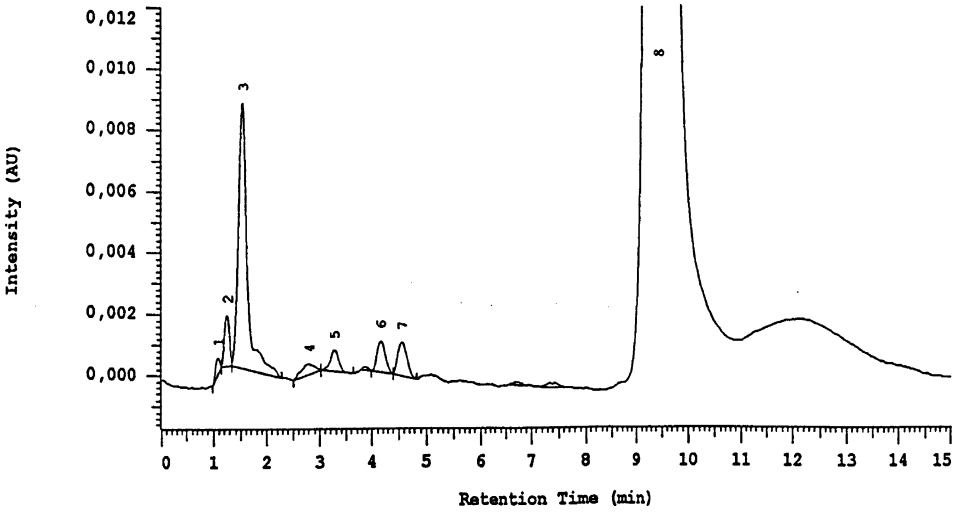

Fig. 4. Chromatogram recorded for the model solution. Peaks denoted 1–5 were assigned to the components of placebo; 6 and 7, to impurities E and D; 8, to glyceryl nitrate.

Table 1
The results of determination: concentrations (%) of glyceryl trinitrate and impurities D and E in reference ointment and Nitrocard ointment

	Reference ointment			Nitrocard ointment		
	Glyceryl trinitrate (%)	Impurities (%)		Glyceryl trinitrate (%)	Impurities (%)	
		D	E		D	E
	2.13	–	0.27	1.84	0.12	0.53
	2.12	0.41	0.22	1.84	0.13	0.55
	2.12	0.54	0.38	1.84	0.14	0.59
	1.93	0.38	0.24	1.86	0.14	0.52
	2.01	0.35	0.25	1.87	0.16	0.51
	1.97	0.30	0.24	1.87	0.14	0.40
	2.11	0.35	0.35	1.85	0.15	0.53
	2.11	0.35	0.35	1.85	0.15	0.50
	2.11	–	0.27	1.86	–	0.53
x	2.068	0.383	0.276	1.853	0.141	0.518
S	0.076	0.077	0.059	0.012	0.012	0.051
μ	0.008	0.009	0.033	0.008	0.009	0.033
%R.S.D.	0.7	8.8	9.9	0.7	8.8	9.9

E, 2-hydroxypropane-1,3-diyl dinitrate; D, (2*RS*)-3-hydroxypropane-1,2-diyl dinitrate; x, mean; S, standard deviation; μ, confidence interval for $P = 95\%$; R.S.D., relative standard deviation.

tage and confidence interval (μ) at significance level $\alpha = 0.05$.

The accuracy of the method was expressed as percentage recovery obtained when analysing reference drug. The recovery is 100.8% for glyceryl trinitrate and 99.0 and 95.1% for impurities D and E, respectively.

The results of determination for reference drug listed in Table 1 are consistent with the declared constituent concentrations and feature of repeat-

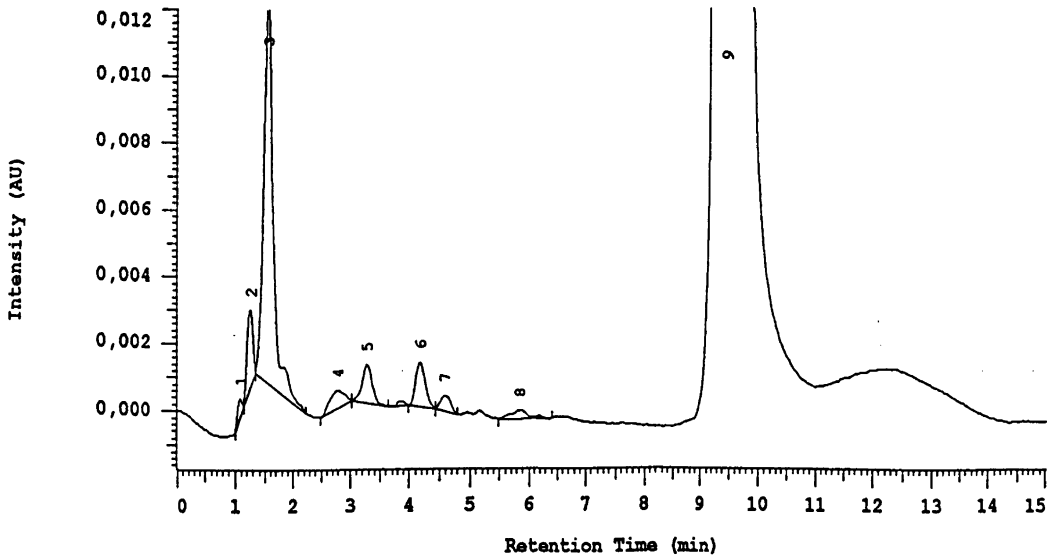

Fig. 5. Chromatogram recorded for the solution of Nitrocard ointment. Peaks denoted 1–5 and 8 were assigned to the components of placebo; 6 and 7, to impurities E and D; 9, to glyceryl nitrate.

ability and low scatter proven by statistical analysis.

The suitability of the method was checked under conditions mentioned above by determining active substance and impurities in Nitrocard. As in the case of the model ointment, chromatograms recorded for Nitrocard ointment revealed no interference between the peaks of glyceryl trinitrate, impurities D and E, and the peaks assigned to the matrix (Fig. 5).

Some differences refer only to quantification of individual components. The results obtained for both ointments differ; these differences, however, are within the accepted pharmacopeian limits. The results are presented in Table 1.

The results of determination for glyceryl trinitrate and impurities show also repeatability and low scatter around the mean value. The results are within tolerances for such drugs. The concentration of impurity D (0.141%) is lower that of impurity E (0.518%).

4. Conclusions

A HPLC method for determining glyceryl trinitrate and impurities D and E in ointment Nitrocard was developed. The results obtained in the validation process and drug analysis are encouraging and indicate suitability for routine tests. Under established conditions there are no interference from vehiculum. In addition, good separation of constituents under consideration is achieved. The method features of good precision and accuracy as confirmed by the results presented in Table 1.

The linearity is maintained within a wide range of concentrations. The limit of detection varies from 70 to 90 ng ml^{-1}, thus indicating its significance in low concentration applications such as determination of impurities.

The fact that the drug under examination contains impurities in the form of dinitro glycerol derivatives indicates the need for further investigations to develop an appropriate level control method.

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