Validated, Stability-Indicated Quantitative Purity Test for Triethylenetetramine Tetrachlorhydrate by Automated Multiple Development

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Abstract

There is a monography of Triethylenetetramine dichlorhydrate (Trientine) in the United States Pharmacopeia. But neither the base nor the salts di- or tetra-chlorhydrate are in the European Pharmacopeia. Triethylène tetramine tetrachlorhydrate, used by AGEPS now as matural, is more soluble then triethylene tetramine dichlorhydrate. It is administred to patients with Wilson's disease, which results from a congenital lack of the copper metabolism. A quantitative purity test of this drug by automated multiple development high-performance thin-layer chromatography is developed and validated. The validation parameters tested are specifically characterized by retention factor, linearity, limits of detection and quantitation of several nanograms, reliability, and accuracy. To determine impurities, the monography of triethylenetetramine dichlorhydrate in the American Pharmacopeia is tested. This method in classic developing tank requires two mobile phases and is not quantitative. Assays in high-performance liquid chromatography with a different column and mobile phase did not give good results for the separation of impurities. Thus, it is not possible to perform comparative validation of the separation of the impurities. Only the assay of triethylenetetramine with potentiometer detection has been validated.

Introduction

Capsules containing 150 mg of triethylenetetramine (TETA) tetrachlorhydrate (4 HCL) are part of the orphan drugs arsenal. The currently admitted criteria for this class of drugs are a prevalence of less than or equal to 0.05%, the absence of an alternative therapy, or a life-threatening situation (1).

This drug is used as second intent in Wilson's disease (after a failure of or intolerance to D-penicillamine). The disorder is characterized by defective copper elimination, resulting in the accumulation of the metal, first in the liver and then in many organs (brain, eye, etc.). If the disease is not treated in time, its course is systematically fatal. This chronic disease requires treatment for the patient's entire life.

The aim of the present work was to guarantee the quality and safety of a starting material intended to be used as a medicine preparation after its pharmaceutical preparation.

The impurities to be detected in the starting material are described in the United States Pharmacopoeia (2). An automated multiple development (AMD) method is used for this detection.

Experimental

To determine impurities, the monography of TETA dichlorhydrate in the American Pharmacopeia was tested. This method in classic developing tank requires two mobile phases and is not quantitative.

Assays in high-performance liquid chromatography (HPLC) with differents columns and mobile phases did not give good results for the separation of impurities; thus, it was not possible to perform comparative validation of the separation of the impurities. Therefore, an AMD method was used for separation of these impurities.

Only the assay of TETA 4 HCl by complexometry with copper in the presence of sodium hydrogenocarbonate with potentiometric detection, has been validated.

Reagents

All reagents used were analytical grade. Methanol and acetonitrile were from Carlo Erba (Milan, Italy). Ammonia (25%) used to prepare the mobile phase was obtained were from Merck (Darmstadt, Germany). Ninhydrin to prepare the dilute solution R1 according to the European Pharmacopoeia (3) was obtained by Fluka (Buchs, Switzerland). TETA 4 HCL references were obtained from Seratech (Gottingen, Germany) and Aldrich (Milwaukee, WI) as were impurity references: diethylenetriamine (impurity B), 1-(2-aminoethyl) piperazine (impurity C), tris (2-aminoethyl) amine (impurity D), and non-fluorescent (10 × 20-cm) silica gel 60 high-performance thin-layer chromatography (HPTLC) plates were from Merck.

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Equipment

The automatic sample applicator (TLC applicator AS 30), the densitometer for quantitative analysis (CD60 Densitometer), and the videodensitometry system were all purchased from DESAGA GmbH (Heidelberg, Germany). The visualization reagent spraying system was provided by Merck. The AMD chamber and the hot plate were from Camag (Muttenz, Switzerland)

Method

Preparation of impurities solutions

Standard A was prepared by dissolving an accurately weighed quantity of TETA 4 HCL in methanol to obtain a solution containing 15 mg/mL.

Standard B was prepared by dissolving an accurately weighed quantity of diethylenetriamine in methanol to obtain a solution containing 1.0 mg per mL. Then 10 mL of this solution was transferred to a 100-mL volumetric flask, diluted with methanol to volume, and mixed.

Standard C was prepared by dissolving an accurately weighed quantity of 1-(2-amino ethyl) piperazine in methanol to obtain a solution containing 1.0 mg/mL. Then 10 mL of this solution was transferred to a 100-mL volumetric flask, diluted with methanol, and mixed.

Standard D was prepared by dissolving an accurately weighed quantity of tris(2-amino ethyl) amine in methanol to obtain a solution containing 1.0 mg per mL. Then 10 mL of this solution was transferred to a 100-mL volumetric flask, diluted with methanol, and mixed.

Sample application

Bands were 5 mm wide, separated by a 10 mm space. The spraying rate was 5 µL/min for a volume of 3 µL per deposit. If several deposits were superimposed, there was a 10 s wait between each application.

Development

The AMD chamber is used to carry out automatic migration. In comparison to a conventional closed chamber, it avoids the need to visually monitor during development. All parameters can be programmed (4), such as: preparation, the chamber is saturated with 25% ammonia; development distance, 18-step isocratic development with a 3 mm increment; and predetermined drying time, after each step, drying time was 3 min for the first four steps and 4 min for the rest.

After the completion of development, the plate was dried and protected in the chamber until it removed (Table I).

Table I. AMD-Step Gradient

Step	Time(mn)	Step	Time(mn)
1	0.2	10	3.1
2	0.5	11	3.5
3	0.8	12	4.0
4	1.0	13	4.5
5	1.3	14	5.0
6	1.6	15	5.7
7	1.9	16	6.5
8	2.3	17	7.2
9	2.7	18	8.0

Mobile phase

The mobile phase was a freshly prepared mixture of methanol–ultrapure water–acetonitrile (5:1:4, v/v/v) (5).

Visualization

After development, the plate was removed from the chamber and dried in an oven at approximately 100°C for 15 min. This parameter is important for the maximal elimination of the mobile phase from the stationary phase. Visualization was performed by spraying with dilute ninhydrin R1 in a ventilated hood. After spraying, the plate was placed on a 105°C hot plate for 5 min. Spots of the different impurities and of the TETA 4 HCL were violet on a white to slightly yellow background.

Results

Quantitation by densitometry

Scanning photodensitometry was then used to measure the intensities of spots at 510 nm using plate background as a reference (4,5). Peak area was calculated with the CD 60 software included in the quantitation system. The software calculates a linear regression coefficient that does not pass through the

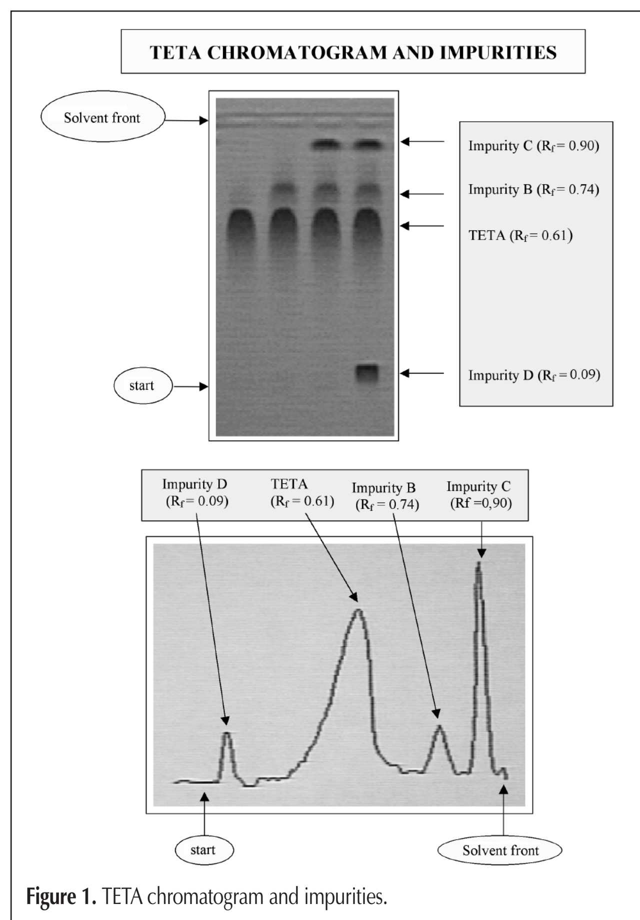

Figure 1. TETA chromatogram and impurities.

origin (particularly of planar chromatography). The quantity of impurities deposited is determined versus the measured peak area.

Validation of the method

Validation of the assay method by HPLC described in the literature (6,7) was used. Validation was based on verifying several criteria: specificity, sensitivity (limits of detection and quantitation), linearity, reliability, and accuracy. For reasons of simplification, the impurities are called B, C, and D (as described in the Reagents section)

Specificity

The TLC method developed enabled the separation of TETA, 4 HCL, and three impurities resulting from the synthesis process (Figure 1).

The following retention factor (R_f) values are obtained: TETA (4 HCL), R_f = 0.61 (CV = 0.68%); impurity B, R_f = 0.74 (CV = 0.52%); impurity C, R_f = 0.90 (CV = 0.37%); and impurity D, R_f = 0.09 (CV = 3.63%)

Sensitivity

The limits of detection were 27.5 ng for impurity B, 7.5 ng for impurity C and 3.5 ng for impurity D.

The limits of quantitation was 90.75 ng for impurity B, 24.75 ng for impurity C, and 11.55 ng for impurity D.

Table II. Linearity Parameters

	Impurity B	Impurity C	Impurity D
Correlation coefficients			
Day 1	0.986	0.997	0.996
Day 2	0.983	0.999	0.991
Day 3	0.986	0.995	0.996
Linearity intervals			
	96 à 192 ng	24 à 120 ng	12 à 96 ng
Regression linears			
	$y = 4.9x - 285$	$y = 2.37x + 18.44$	$y = 4.63x + 74.97$

Table III. Reliability Parameters

	Variation coefficient of repeatability (%)	Variation coefficient of intermediate reliability (%)
Impurity B	4.94	5.63
Impurity C	4.44	5.31
Impurity D	4.56	6.44

Table IV. Accuracy

	Mean recovery (%)	Confidence intervals (%)
Impurity B	102.16	97.57 à 104.75
Impurity C	103.31	100.87 à 105.75
Impurity D	106.55	103.6 à 109.5

Linearity

Linearity was determined using a 5-point range prepared just before use. The test was run over 3 days at one series per day (Table II). The correlation coefficients, the linearity intervals, and the linear regression were determined for each impurity.

Reliability

Reliability expresses the degree of consistency between a series of determinations made on several aliquots of the same homogeneous sample in defined conditions.

Determining reliability is the same as determining the repetability of the method, established by seven assays of the mid-point of the range and intermediate reliability with the same range point over three days.

After verifying the homogeneity of variances between the different days, the coefficients of variation of repetability were calculated for each of the three impurities (Table III).

Accuracy

Accuracy was conducted with linear regression data (i.e., it was determined by carrying out three series of seven applications of a reference solution of each of the impurities in the mid-point range). Mean recovery and confidence intervals determined for each of the impurities are listed in Table IV.

Discussion

The choice of planar chromatography was a good one because this technique easily gets around the detection problem related to the lack of absorbance of TETA 4 HCL and of the three impurities in the UV by use of a visualization reagent (ninhydrin solution R1).

Statistical parameters determined during validation were entirely acceptable for the detection and quantitation of the three impurities resulting from the synthesis process. Linearity

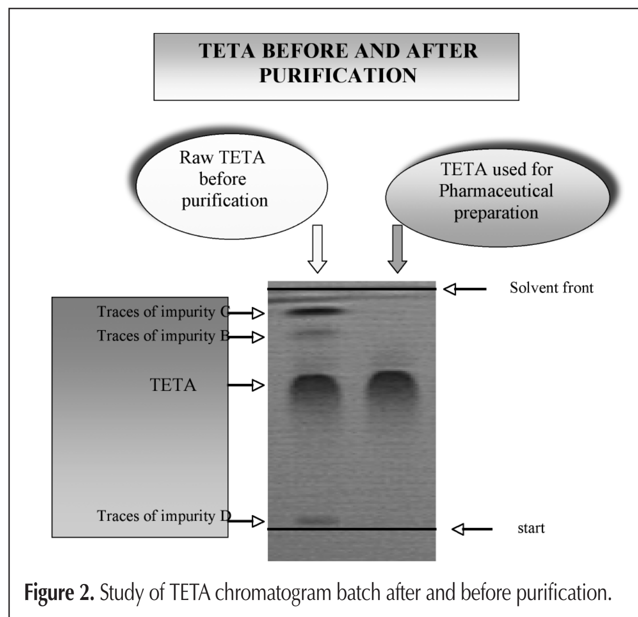

Figure 2. Study of TETA chromatogram batch after and before purification.

was good, in particular for impurities C and D (correlation factor > 0.99) and somewhat less for impurity B (correlation factor > 0.98). This results from the greater diffusion of the spot that reduces its sharpness and, thus, leads to a relatively high limit of detection (approximately 27 ng), while those of impurities C and D were 7.5 and 3.5 ng. Reliability and accuracy were acceptable, even though the technique tended to slightly overestimate the quantities of impurities applied to the plate. The regression lines calculated on days 1, 2, and 3 differed by their slopes and Y-intercepts.

The observed differences show that the method is not very rugged and is entirely predictable in planar chromatography. Even so, this drawback can be addressed if a calibration range is run with each HPTLC assay plate.

The method was used to detect the synthesis of impurities in a batch of TETA 4 HCL destined to manufacture the medicinal preparation; none of the three impurities could be detected. When the same starting material was analyzed before purification, however, impurities were detected, in particular, 1-(2-aminoethyl)piperazine. Its level was high, exceeding the high point of the calibration range (> 0.8% of the unpurified starting material). Traces of diethylenetriamine were detected, but could not be quantitated (Figure 2).

Conclusion

The method developed was valid for purity testing, a stability indicating assay. Validation of this method showed that it is specific, linear, reliable, and accurate (although slightly overestimated), but the sensitivity was moderate for one of the three impurities sought (diethylenetriamine). This methodology is well suited for monitoring the purification of manufactured batches in order to comply with ICH recommendations concerning levels of impurities (8).

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