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EXTENDED RELEASE TABLETS CONTAINING CO-PROCESSED DRY BINDERS AND TRAMADOL HYDROCHLORIDE – DETERMINATION OF THE CONTENT UNIFORMITY

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An automated method for the extended release tablet processing and determination of tramadol hydrochloride in matrix tablets containing co-processed dry binders has been developed by using a bench-top robotic system connected with HPLC system. The automated operations include (i) disintegration steps of the dosage form, (ii) homogenisation, (iii) setting time, (iv) filtration, (v) dilution, and (vi) injection of a sample into the HPLC vials or chromatographic system. The method developed has been optimized and validated; recoveries of spike tests being found in the range of 97.8-99.6 %. The relative standard deviation representing the precision of the method was 1.02 % for tramadol

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hydrochloride in tablets with Disintequik™ MCC 25 and 1.15 % for tramadol hydrochloride in tablets with Prosolv® SMCC 90. The validated method was applied to the determination of the target substance in samples of two commercial pharmaceutical formulations: Tralgit SR 100 and Tramadol Retard Actavis 100 mg.

Introduction

Tramadol hydrochloride (TH) is a synthetic opioid analgesic available in various commercial drugs in the form of drops, capsules, formulations for control release (oral use) or suppositories (rectal use) [1]. Many new drug formulations with TH especially controlled release-drug systems are still developed and marketed. Manual methods for the determination of a content uniformity of TH tablets already exist but manual processing of solid samples involves numerous error-prone operations (e.g. sample weighing, solvent addition, homogenisation, extraction, filtration, dilution, and transfer to analysis devices) and, accordingly, the results of analysis dependent, among others, on experimental skills and experiences. Automated methods offer advantages as electronic documentation of sample analysis, higher precision and reproducibility; all achieved by minimizing of the errors caused by human factor, reduced solvent waste, and lower time demands. In addition to these benefits, automated sample preparation also enhances laboratory productivity by simplifying resource allocation for repetitive operations.

The goal of this paper was to develop and validate an automated method for the determination of the content uniformity of tablets with extended release of tramadol hydrochloride using Tablet Processing Workstation (TPW III, Sotax) with HPLC system (see Fig. 1). TPW is the bench-top system controlled by computer and offering the options for working up with samples in the tablet form. All operations and generated data (including sample weight, weight of added solvents, homogenizer speed, etc.) are documented by the software which allows one to reveal possible errors [2-4]. Densities of all solvents have to be known prior to sample preparation, because the solvent addition is controlled gravimetrically [5]. Gravimetric indication of liquid handling operations ensures accuracy and precision of the sample dilution process.

The first part of this work is focused on the method development based on extracting the active substance from the tablet matrix. Key parameters, such as the probe speed, number of pulses, homogenisation time, filtration, and dilution were optimized. The second part is then focused on validation of the method; the key parameters being the system linearity, precision and accuracy, reproducibility, and comparison between the manual and automated procedures. Finally, the last sequence of this work is devoted to the applicability of the (new) method in analysis of commercial drugs.

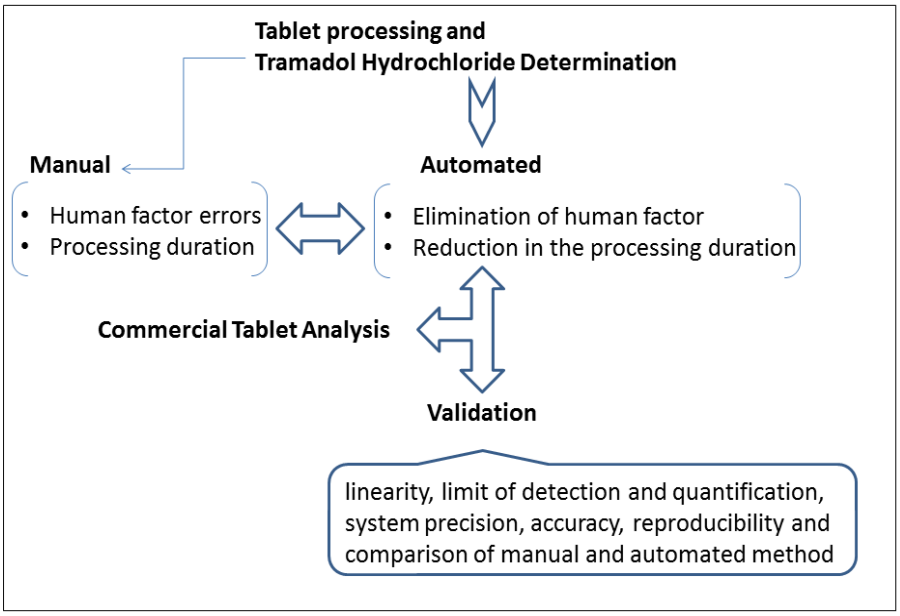

Fig. 1 Scheme of the development of new automated method

Experimental

Apparatus

Sample preparation and analysis were performed using the Tablet Processing Workstation (model TPW III, with software version 2.0; Sotax, USA) connected with an HPLC system (model Agilent 1100; Agilent Technology, Waldbronn, Germany) and using the column C18, 150×4.6 (ZORBAX Eclipse XDB 5µm; Agilent, Santa Clara, USA). Chromatographic data were analysed and stored by means of chromatography data system (Empower Pro Software, version 5.00, Empower Software Service Pack SP-D; Waters, Milford, USA). Samples tablets (with hydrophilic matrix) were prepared using a material testing equipment (model Zwick / Roell, T1-FRO 50 TH.A1K; Zwick, Germany).

Chemicals and Reagents

Tramadol hydrochloride [European Pharmacopoeia (EP) Reference Standard, Sigma-Aldrich] was used for tablet preparation and for all measurements as the standard of the active substance. The standard solutions were prepared by dissolving 20 mg TH reference standard in 100 ml 0.1 M HCl or in deionized water. Acetonitrile (for HPLC, ≥ 99.9%, Sigma-Aldrich) and trifluoroacetic acid (for HPLC, ≥ 99%, Sigma-Aldrich) were used for HPLC determination of TH.

Hypromellose Methocel™ (product K100M Premium CR; Colorcon, Germany) was used as controlled release agent forming hydrophilic matrix system. Disintequik™ MCC 25 (Kerry, USA) and Prosolv® SMCC 90 (JRS PHARMA; KG, Germany) were the co-processed dry binders of choice, whereas magnesium stearate (Acros Organics, USA) served as lubricant.

Tablet Samples

Two different types of matrix tablets (sample A and B) with extended release of TH were prepared by direct compression method using a material testing equipment T1-FRO 50 TH.A1K Zwick/Roell in combination with a special die with lower and upper punch. The tablets were of cylindrical shape without facets measuring 13 mm in diameter and weighing 0.5 ± 0.0010 g; compression force of 6 kN was employed.
Composition of sample A: 100 mg tramadol hydrochloride, 150 mg hypromellose Methocel™ K100M Premium CR, 245 mg Disintequik™ MCC 25 and 5 mg magnesium stearate.
Composition of sample B: 100 mg tramadol hydrochloride, 150 mg hypromellose Methocel™ K100M Premium CR, 245 mg Prosolv® SMCC 90 and 5 mg magnesium stearate.

Two different types of commercial tablets (lipophilic and hydrophilic) with extended release of TH were used for verification of the method. Tralgit SR 100 (Zentiva, Slovak Republic) was used as a representative sample of the lipophilic matrix tablet, Tramadol Retard Actavis 100 mg (Actavis Group hf., Iceland) served as a tablet sample with the hydrophilic matrix.

Manual Sample Processing

One tablet was crushed in mortar and transferred into a 200 ml volumetric flask. Then, 150 ml 0.1 M HCl or deionized water (for optimisation of manual sample processing) was added and an ultrasonic bath used for total disintegration of the tablet (for 30 min, approximately). After stirring the prepared solution for 3 hours, the sample volume was made up to 200 ml with 0.1 M HCl. Then, a portion of solution was filtered using a glass microfiber filter 0.45 μm (Whatman®) and diluted 5× by deionized water. The resultant clear solution was injected into the chromatographic system.

Automated Sample Processing Using TPW III

Tablets were put into a sample tube manually and the following operations managed automatically. The sample was weighed and transferred into the homogenisation vessel with 200 ml 0.1 M HCl as the solution for extraction. The solvent was dispensed gravimetrically. For homogenisation, a pulse for 80 s at 7000 rpm was used with a probe movement of 1cm above the bottom of the vessel and back, followed by another pulse for 360 s at 8000 rpm without probe movement. After a quiet time for 30 s, 8 ml of solution was filtered using a 0.45 μm glassy fiber filter, volume of 0.8 ml was taken and diluted 10× with deionized water. This solution was stirred for 60 s and collected to an EasyFill modul into the HPLC vials. The whole process has taken about 30 min., including clean-up process, and is summarized in Table I.

Table I Summary of the final TPW method: Operational sequences

Step	Operation
1	Put one tablet into a sample tube and weigh the sample.
2	Transfer 200 ml 0.1 M HCl into the homogenisation vessel.
3	Transfer the tablet sample into the homogenisation vessel.
4	Disperse the tablet using 1 pulse for 80 s at 7 000 rpm with probe movement.
5	Disperse the tablet using 1 pulse for 260 s at 8 000 rpm
6	Settle for 30 s (quiscent period).
7	Filter 8 ml of dispersion using 0.45 μm GF, take 0.8 ml and dilute 10×.
8	Stir for 60 s.
9	Inject the diluted sample solution in the HPLC system and perform the determination of TH.
10	End the procedure and clean TPW III system.

HPLC Analysis

HPLC analyses were performed in accordance with European Pharmacopoeia 8th [6]. Mobile phase was prepared from trifluoracetic acid and water (2 ml CF_3COOH + 998 ml H_2O) and mixed with acetonitrile (70:30, v/v). An isocratic method was applied with a flow rate of 1 ml min^{-1} and column temperature of 37 °C. The injection volume was 20 μl. The wavelength of 271 nm was used for UV detection of TH.

TPW Method Validation

The method validation involved the following key criteria: the linearity of chromatographic system and TPW method, limit of detection and quantification, precision and accuracy of the system, reproducibility, and comparison of manual with automated method.

Linearity of the Chromatographic System. The linear range was defined by the calibration curve method. Solutions of TH in 0.1 M HCl were injected (in triplicate) into the HPLC system in a concentration range from 6.25 to 200 mg l^{-1}. The resulting calibration curve was evaluated from the dependence of the peak area on TH concentration and evaluated by linear regression. Limit of detection (*LOD*) and quantification (*LOQ*) were calculated according to the relations $LOD = 3h_n/m$ and $LOQ = 10h_n/m$, respectively, where h_n is noise of the base line and m is the slope of calibration curve (expressed as the peak height dependence on the drug concentration).

Linearity of the Method. This related parameter was evaluated by analysis of increasing amount of powdered sample A and sample B. For tramadol hydrochloride, six sample weights have been determined in triplicate and correlation between the sample weights and TH content was investigated.

Precision of the Chromatographic System. Precision of the HPLC system was determined from evaluation of peak areas and retention times after injecting the TH standard solution of 0.1 mg ml^{-1} in seven replicates (7×) during one single day (intra-day variation) and 7× in seven days (extra-day variation). Precision was expressed as the relative standard deviation (RSD) of the retention times and peak areas. Similarly, a comparison of the precision between different HPLC systems (Agilent, Waters) was also evaluated.

Accuracy and Reproducibility of the Method. Accuracy and reproducibility of TPW method were ascertained from the recovery study, when TH tablets (as a sample A and sample B) were spiked with various amounts of TH and analyzed using the TPW method. Tablets were milled prior to analysis and 125 mg powdered form (with the content of TH of 25 mg) used for each recovery experiment. This amount of sample was spiked with TH at different levels (addition of 25, 50, 75, 100, and 125 mg TH). Six spiked samples at each concentration level ($n = 6$) were then analyzed.

Comparison of the Manual and Automated Method. The last key criterion for validation of the automated method was comparison of the results of content uniformity obtained by manual procedure and the TPW method in the automated regime. The mean value, standard deviation (*SD*) and an interval of lower and higher value (L–H) were defined for both methods. Using each method, six tablets of sample A and six tablets of sample B were analyzed.

Results and Discussion

Optimisation of the Manual Method

Basic experimental procedure for the manual method was already optimized during the preliminary experiments and the choice of the proper solvent (200 ml deionized water or 0.1 M HCl) due to its effect on sample homogenisation confirmed for tablets with the extended release of TH. Therefore, a series of six solutions with deionized water and six solutions with 0.1 M HCl as the solvent were prepared according to the procedure described above (see Manual Sample Processing) in order to determine the content of TH. It was found that the sample homogenisation using deionized water as the solvent had been insufficient and the mean value for TH content was only 91.75 % (with *SD* 1.93 %).When 200 ml 0.1 M HCl was used for the sample preparation, the tablet disintegration was more effective and 99.09 % TH (with *SD* 1.01 %) determined. Thus, for the sample preparation by the manual method described above, 200 ml 0.1 M HCl was used as the solvent. The results, namely, the mean value, standard deviation (*SD*) and an interval from lower to higher value (*L-H*) for solvent optimisation are summarized in Table II.

Table II Optimisation of the solvent for manual method (sample A). Mean values, standard deviation (*SD*) and interval from lower to higher value (*L-H*) from analysis of six samples ($n = 6$)

Solvent	Mean mg tablet^{-1}	*SD* mg tablet^{-1}	*L-H* interval mg tablet^{-1}
Deionised H_2O	91.75	1.93	89.66-92.56
0.1 M HCl	99.06	1.01	97.51-100.84

Development of TPW Method

The automated method described in Automated Sample Processing Using TPW II has been developed (using sample A) in an effort to use it for the determination of the content uniformity of newly prepared tablets with the sustained release of TH. Initial parameters of the TPW method were chosen based on preliminary experiments.

During the method development, these experimental parameters were optimized: type of the solvent, sample homogenisation (with homogenisation time) and the rinsing step. In initial TPW method, the homogenisation time for 60 s was applied. As seen in Table III, the mean value of determined amount of TH using homogenisation time for 60 s is only 90.33 % (with *SD* 2.20 %); therefore, the

homogenisation time had to be extended.

Using homogenisation time of 360 s, the mean amount of TH increased to 91.84 % (with *SD* 1.92 %). In the next step, effect of the solvent on the sample disintegration and homogenisation was studied by optimizing the sample extraction adapted to the tablets with the extended release of TH. During preliminary TPW experiments, it was found that TH tablets without modified release had readily disintegrated and could be dissolved in 200 ml deionized water. In initial TPW method with the extended release tablets, deionized water was used as the solvent, but it was found that the tablet disintegration in this solvent had not been complete, the respective recovery of TH was low, and therefore, water replaced with 0.1 M HCl as in the procedure for the manual method. By exchanging the solvent, the TH recovery increased to 92.95 % (*SD* 0.64 %). For this reason, 0.1 M HCl (200 ml) had to be used as the solvent for the quantitative dissolution and homogenisation of tablets with the extended release of TH.

The key parameter for optimization was also the cleaning step. During the method development, it became obvious that different dilution had to be used due to the cleaning process. A portion of 0.8 ml of the sample solution was taken and diluted 10× with deionized water. It was also necessary to optimize the volume of the solvent for the washing power of the transfer path between the tubes and HPLC vials in an EasyFill collection modul. As presented in Table III, the concentration of TH using one rinsing step (1×1.5 ml) was about 2 % lower than the content in the test tube before the transfer path. It was confirmed that the minimum volume of the solution for the quantitative sample transferring between the tubes and HPLC vials was 2×1.5 ml. Under such conditions, the concentration of TH during the transfer between the tubes and HPLC vials did not decrease. The results from the development and optimization of TPW method, including the *L-H* interval, the mean value, *SD* and *RSD* are shown in Table III.

Table III Development and optimisation of the TPW method (sample A, $n = 6$)

	Mean mg tablet^{-1}	*SD* mg tablet^{-1}	*RSD* %	*L-H* interval mg tablet^{-1}
Manual method	99.09	1.01	1.6	97.51-100.84
TPW, initial stage	90.33	2.20	2.44	89.00-92.88
Homogenisation, 360 s	91.84	1.92	2.09	89.66-93.30
0.1 M HCl (solvent)	92.95	0.64	0.69	92.49-93.40
1× rinsing	95.97	0.85	0.89	95.00-96.19
2× rinsing	98.88	1.14	1.15	97.47-99.77
TPW final stage	99.22	1.35	1.02	97.33-100.70

Table IV System presision. Intra-day (A) and extra-day (B) variation in the peak areas and retention times of TH standard solution, precision of the different HPLC systems ©

A) Intra-day precision

Replicate	Retention time, min	Area, AU
1	2.729	1 904 233
2	2.729	1 906 289
3	2.731	1 906 602
4	2.729	1 906 332
5	2.730	1 906 734
6	2.729	1 906 663
7	2.727	1 909 430
Mean	2.729	1 906 612
SD	0.0012	1 517
RSD, %	0.044	0.080

B) Extra-day precision

Replicate	Retention time, min	Area, AU
1	2.786	1 905 611
2	2.731	1 904 171
3	2.729	1 906 611
4	2.765	1 907 276
5	2.768	1 905 610
6	2.721	1 906 233
7	2.719	1 908 917
Mean	2.746	1 906 347
SD	0.027	1 493
RSD, %	0.98	0.078

Table IV – Continued

C) HPLC systems

Replicate	Retention time, min	Area, AU
1 (Agilent Technologies)	2.773	1 907 423
2 (Waters)	2.912	1 899 651
3 (Agilent Technologies)	2.729	1 902 363
Mean	2.805	1 903 146
SD	0.095	3 945
RSD, %	3.39	0.21

Validation of the TPW Method

The key criteria for method validation as the linearity, limit of detection and quantification, precision and accuracy of the system, reproducibility, and comparison of manual and automated method arrangement were determined.

Linearity of the chromatografic system. Linear range of the chromatografic system was determined by analysis of the standard solutions of TH and confirmed in a range from 6.25 to 200 mg l^{-1} (6.25-200 % of the nominal amount of TH in tablets). The dependence of the peak areas on the concentration was evaluated using linear regression analysis, resulting in Eq. $y = 7042.1\ x - 5014.3$, $R^2 = 0.9997$, where y corresponds to the peak area and x expresses concentration of TH (in mg l^{-1}). For both samples, the linear range determined was found to cover the concentration interval expected for the content of TH during analysis of tablets. The values of *LOD* and *LOQ* (calculated according to the Eq. in TPW Method Validation) were estimated to be about 5.6 μg l^{-1} and about 18.8 μg l^{-1} TH, respectively.

Linearity of the method. The linearity of the TPW method (correlation between sample weight used for analysis and TH content) was evaluated for both samples. Results are summarized in Table V. It can be seen that the correlation is linear over a wide range of tablet weights and TH content. The linear range covers common controlled release tablet weights and corresponding TH content.

Precision of the chromatographic system. HPLC system precision was evaluated from the peak areas and retention times after injecting the TH standard solution (0.1 mg ml^{-1}) 7× during one single day (so-called "intra-day variation") and 7× in seven consecutive days ("extra-day variation"). The intra-day system precision

Table V Linearity of the TPW method for sample A and sample B

Sample	Linear range g TH per sample*	Slope ± *SD*	Intercept ± *SD*	R^2
A	0.025-0.15	0.20 ± 0.0028	–0.835 ± 0.043	0.9997
B	0.025-0.15	0.19 ± 0.0031	–1.064 ± 0.077	0.9998

*A sample content of 0.025 g TH corresponds to 0.125 g powdered tablet used for analysis;

defined as the relative standard deviation (*RSD*) of the retention times and the peak values rounded areas of TH was 0.044 % and 0.080 %, respectively (see Table IVA). Similarly, the extra-day precision expressed as the *RSD* of the retention times and the peak areas of TH yielded values 0.98 % and 0.078 %, respectively (Table IVB). Also, the precision between different HPLC systems was evaluated from retention times and the peak areas after injecting the standard solutions of TH. As seen from Table IVC, a high precision (with *RSD* 3.39 % and 0.21 %, respectively) was confirmed.

Table VI Accuracy and reproducibility. Recovery of TH at 5 concentration levels, 6 measurements for each sample level ($n = 6$)

A) Sample A

TH added mg	*L-H* mg	TH recovered ± *SD* mg	Recovery ± *SD* %
25	48.81-49.98	49.40 ± 0.49	98.9 ± 0.9
50	72.21-74.93	73.87 ± 0.99	98.5 ± 1.3
75	97.33-100.05	98.97 ± 0.99	99.0 ± 0.9
100	123.26-124.98	124.41 ± 0.69	99.5 ± 0.5
125	148.28-150.03	149.44 ± 0.69	99.6 ± 0.4

B) Sample B

TH added mg	*L-H* mg	TH recovered ± *SD* mg	Recovery ± *SD* %
25	48.33-49.49	48.91 ± 0.48	97.8 ± 0.9
50	72.93-74.82	74.11 ± 0.65	98.8 ± 0.8
75	97.99-99.71	98.69 ± 0.73	98.7 ± 0.7
100	123.26-124.98	124.25 ± 0.63	99.4 ± 0.5
125	148.11-149.98	149.29 ± 0.72	99.5 ± 0.5

Accuracy and reproducibility of the method. These parameters were evaluated from the recovery study, when TH tablets (denoted as sample A and sample B, respectively) were spiked with various amount of TH and then analysed using TPW method; the results being statistically evaluated. As seen from the results of experiments with spiked concentrations (Table VI), the mean recovery of TH was in a range of 98.5-99.6 % for sample A and 97.8-99.5 % for sample B of the predicted value. The *SD* = 1.3 % or less for both samples has then confirmed an adequate accuracy of the method; the accuracy of TPW method being comparable for all the samples and independent of the different amount of TH.

Content Uniformity: Comparison of Manual and Automated Method. The final step of the validation process was to evaluate the content uniformity in both samples A and B using the manual procedure and TPW method; the corresponding results being expressed as the mean, RSD, and *L-H* interval gathered in Table VII. The values of recovery of TH show good agreement between the automated and manual methods, whereas the values of *RSD* (e.g., for sample A, 1.02 % using TPW and 1.36 % for manual regime) indicate a higher precision of the TPW method.

Table VII Comparison of the manual and TPW method ($n = 6$)

	Sample A		Sample B	
	Manual method	TPW method	Manual method	TPW method
TH analysed, mg tablet^{-1}	99.09	99.22	99.12	99.19
RSD, %	1.36	1.02	1.46	1.15
L-H interval, mg tablet^{-1}	97.51-100.84	97.33-100.70	97.17-100.91	98.34-100.80

Table VIII Quantification of Tramadol hydrochloride in commercial tablets ($n = 6$)

Sample	Declared amount of TH mg	TH analysed mg	*RSD* %
Tralgit SR 100	100	98.54	1.27
Tramadol Retard Activis 100 mg	100	98.54	1.08

Analysis of Commercial Formulations (Tablets)

Two types of commercial tablets with TH were processed by using the validated TPW method and the content uniformity was evaluated. Because of the fact that the extended release tablets containing TH and co-processed dry binders are not yet commercially available in the Czech Republic, *Tralgit SR 100* and *Tramadol Retard Actavis 100 mg* (tablets with extended release of TH but without co-processed dry binder) were chosen as model drugs from the commercial sphere. The mean values of the content of TH and the respective RSD are summarized in Table VIII. As seen, the results of TH are in good agreement with the content of TH declared which confirms a high precision of the method in the automated regime. Finally, the results also confirm that the described method can be used for the determination of the content uniformity in different types of extended release tablets.

Conclusion

A new automated method for determination of the content uniformity of tablets with co-processed dry binders, hypromellose, and TH was developed, optimized, and validated. The TPW method includes the disintegration of tablets, homogenisation, filtration, dilution, and injection of samples into the HPLC system. The cleaning step was also key parameter which had to be optimized. As seen from the results of validation, a high precision and accuracy, as well as good reproducibility of the method were confirmed. The results obtained using TPW method have been found comparable with manual method but the automated procedure possesses notable advantages, such as a higher productivity, lower cost per analysis and elimination of errors dependent on the experience of the user / analyst. After validation, the new method could be applied to analyse the commercial tablets having shown itself to be suitable in routine analysis of tablets with the extended release of TH.

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