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Organic elemental analysis: a new universal approach to authenticity/quality control of pharmaceuticals

Expedient and reliable methods for the quantitation of active ingredients in pharmaceutical dosage forms and substances are essential due to the growing problem of counterfeit pharmaceuticals and ineffective quality control in pharmaceutical production.[1] This problem arises from the fact that the active ingredient content is often determined by different methods such as high performance liquid chromatography (HPLC), titrimetry, or potentiometry. Using these methods, different determination conditions are required, which are specific to determining the targeted active ingredient.[2,3] In such cases, the reference samples are used and the calibration is performed for each individual compound. These methods are very time-, labour-, and cost-consuming. As such, they are inappropriate for high-throughput quality control at the site of production.

Organic elemental analysis (OEA) is an alternative, fast and relatively inexpensive method for active pharmaceutical ingredient content determination. We have reviewed the structures of 2500 pharmaceutical compounds which are used as active ingredients in many pharmaceuticals. It was found that approximately 80% of these compounds contain nitrogen, although in the majority of cases the excipients of pharmaceutical dosage forms do not. Thus by determining the nitrogen content it is possible to find out the content of the active ingredient in the respective dosage form, if we determine the nitrogen content in that dosage form. This indirect approach does not require extraction of the active ingredient, sample referencing, or the necessity for accurate and reproducible extract sample injection and calibration. The duration of one analysis depends only on the preparation and weighing of the small part of the grounded solid matrix (or solution) and determination of nitrogen content in that sample by elemental analysis. In addition the determination of nitrogen content by elemental analysis takes only five minutes or less. Therefore, the accuracy and throughput of such determination is much higher than that of the commonly used methods such as HPLC, titrimetry, and potentiometry.

In our opinion, the advantages of such an approach are apparent. Nevertheless, there are no publications in which active pharmaceutical ingredients in dosage forms are quantified by elemental analysis. The purpose of the present work was to study the possibility of fast active ingredient content determination in different pharmaceuticals and substances using an automated elemental analyzer, by means of nitrogen content determination in respective samples.

Helium (99.9999%) and oxygen (99.999%) were obtained from PromGasService (Moscow, Russia). Cystine ($C_6H_{12}N_2O_4S_2$) purchased from ThermoFinnigan (Milan, Italy) was used as the only reference compound for all studied samples. Atropine, sulfanilamide, pentoxifylline, captopril, and BBOT were used as target substances. Target pharmaceutical dosage forms were obtained from different pharmacies in Moscow, Russia.

Organic elemental analyzers model Flash EA 1112 (ThermoFinnigan, Milan, Italy) and model Dumatherm (Gerhardt, Germany) were configured as N-analyzers. They were used for the determination of active ingredient of target pharmaceutical dosage forms and substances by means of nitrogen quantitation.

Analytical balance model MX5 Mettler Toledo, Greifensee, Switzerland was used for the weighing of samples. All samples were weighed and introduced into the OEA autosampler in tin containers (ThermoFinnigan, Milan, Italy).

A 4-point calibration was performed each day prior to the analysis of target pharmaceuticals. The weight of cystine sample (reference compound) was at least 0.5 mg.

Substances or powders from capsules were analyzed directly. In the case of tablets, sample preparation included homogenization (grinding) of 3 tablets of the respective pharmaceutical. Portions of the respective powder (not less than 0.3 mg and not more than 6.5 mg) were analyzed by OEA. The weight of the analyzed sample portion was dependant on the nitrogen content in the active compound molecule and also on the specified content of the active ingredient in its dosage form. It was chosen according to the range of nitrogen content used for the calibration curve in such a manner that the expectable weight of nitrogen in a sample portion was within the minimal and maximal nitrogen weights used for the calibration curve.

In the case of liquid sample analysis, a sorbent was used. Ethanol and methanol evaporated too quickly from the sorbent. Therefore, model solutions used for analysis were prepared in water. Model water solution of the active ingredient with known concentration was placed on the sorbent immediately prior to the analysis. The same sample preparation was used for the liquid pharmaceuticals from ampoules.

The quantitation of the active N-containing ingredient in various pharmaceutical dosage forms and substances using elemental analysis was investigated. Using the proposed OEA approach, analysis of pharmaceutical substances and various pharmaceutical dosage forms was carried out. The data obtained for solids are presented in Tables 1 and 2.

As presented in Table 1, the difference between the specified and experimental values of nitrogen content in substances was within the limits of allowable error. The determined degree of purity was approximately 100%. The overestimated values for the content of active ingredient could be explained with the presence of N-containing impurities.

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Table 1. Results of active ingredient content determination in pure substances by elemental analysis (n = 5, P = 0.95; s_R < 0.6%)

No	Name of compound	Specified nitrogen content in substance, %	Experimental nitrogen content in substance, %	Content of active ingredient in substance, %
1	Pentoxifylline	20.13	20.20 ± 0.03	100.3 ± 0.1
2	Sulfanilamide	16.27	16.34 ± 0.06	100.4 ± 0.4
3	Captopril	6.44	6.49 ± 0.04	100.8 ± 0.6
4	Atropine	4.84	4.87 ± 0.01	100.6 ± 0.2
5	BBOT	6.51	6.54 ± 0.01	100.5 ± 0.1

Table 2. Results of active ingredient content determination in solid dosage forms (tablets and capsules) by elemental analysis (n = 5, P = 0.95; s_R < 3.2%)

No	Active ingredient	Pharmaceutical name (dosage form)	Specified content of active ingredient in dosage form, mg	Experimental content of active ingredient in dosage form, mg
1	Stavudine	Zerit (capsules)	40	39.9 ± 0.3
2	Loratadine	Claritin (tablets)	10	9.9 ± 0.1
3		Clarotadin (tablets)	10	10.0 ± 0.1
4		Erolin (tablets)	10	9.2 ± 0.1
5	Captopril	Capoten (tablets)	25	23.8 ± 0.2
6	Pentoxifylline	Trental (tablets)	100	93.43 ± 0.61
7	Rifampicine	Rifampicine (capsules)	150	145.5 ± 0.9
8	Vinpocetin	Vinpocetin (tablets)	5	5.3 ± 0.1
9		Kavinton (tablets)	5	5.9 ± 0.1
10	Metoclopramide	Metoclopramide (tablets)	10	13.4 ± 0.2
11		Cerucal (tablets)	10	11.0 ± 0.6

As it follows from the data presented in Table 2, the experimental active ingredient content was within the range of allowable error for all tablets except Erolin and Trental. Thus, it could be determined that both Erolin and Trental should be further tested by more sensitive analytical methods. It is potentially possible that the quality of some pharmaceuticals was not adequate given the random nature of their sources.

As seen in Table 3, the difference between the specified and experimental values of nitrogen content in solid substances and respective solutions in the case of pentoxifylline was within the limits of allowable error. The purity degree was approximately 100% in these samples. In the case of captopril, variations in solid substance and solution were slightly different, potentially, due to the lower solubility of captopril in water.

A number of different tablets and ampoules were analyzed, most of them containing the same active ingredients. These results are presented in Table 4. The error of quantitation of the active ingredient in tablets of suprastine and dimedrol was within the allowable range. In the case of analgine, the content was determined as significantly lower than that of tablets and

Table 4. Results of the active ingredient content determination in various tablets and ampoules obtained by elemental analysis (n = 5, P = 0.95; s_R < 3.7%)

No	Active ingredient	Type of pharmaceutical	Specified content of active ingredient in tablet, mg	Actual content of active ingredient in tablet, mg
1	Suprastine	tablets	25	25.8 ± 0.3
	Suprastine	ampoules	20	17.8 ± 0.2
2	Dimedrol	tablets	50	45.0 ± 1.3
	Dimedrol	ampoules	10	9.5 ± 0.3
3	Analgine	tablets	500	439.0 ± 3.2
	Analgine	ampoules	500	392.4 ± 6.6
4	Diclofenac	ampoules	75	74.1 ± 0.6
5	Ortofen	ampoules	75	69.4 ± 1.7

Table 3. Results of active ingredient content determination in pure pharmaceutical substances and their water solutions by elemental analysis (n = 5, P = 0.95; s_R < 2.1%)

No	Name of compound	Specified nitrogen content in substance, %	Actual nitrogen content in substance, %	Content of active ingredient in substance, %
1	Pentoxifylline	20.13	20.20 ± 0.03	100.3 ± 0.1
	Pentoxifylline (solution)	20.13	20.31 ± 0.21	100.0 ± 1.0
2	Captopril	6.44	6.49 ± 0.04	100.8 ± 0.6
	Captopril (solution)	6.44	6.22 ± 0.21	96.6 ± 3.3

Table 5. Comparison of analysis results for substances, tablets and capsules obtained with conventional methods and with elemental analysis (n = 7, P = 0.95)

Substance name	Content of active ingredient in substance specified in analysis certificate, % (quantitation method)	Content of active ingredient in substance determined with elemental analysis, %
Triamterene	100.3 (titrimetry)	100.0 ± 0.3
Hydrochlorothiazide (lot #1)	100.2 (HPLC)	101.1 ± 0.4
Hydrochlorothiazide (lot #2)	100.0 (HPLC)	101.1 ± 0.5
Fosinopril (lot #1)	98.9; 99 (HPLC)	99.4 ± 0.4
Fosinopril (lot #2)	98.4 (HPLC)	99.9 ± 0.4
Captopril	99.1 (titrimetry)	99.1 ± 0.3
Sotalol hydrochloride (lot #1)	99.5 (potentiometry) 99.7 (HPLC) 100.3 (titrimetry)	99.9 ± 0.5
Sotalol hydrochloride (lot #2)	99.9 (HPLC) 103.1 (titrimetry)	99.6 ± 0.5
Stavudine (lot #1)	99.3 (HPLC)	99.9 ± 0.3
Stavudine (lot #2)	100.21 (HPLC)	100.3 ± 0.4
Metoclopramide hydrochloride	99.6 (titrimetry)	99.2 ± 0.6

Pharmaceutical name	Specified content of active ingredient in tablet or capsule, mg	Allowable range of HPLC quantitation results specified in analysis certificate, mg	HPLC quantitation result specified in analysis certificate, mg	Content of active ingredient in tablet or capsule determined with elemental analysis, mg
Sotalex	160	152–168	157.9	158 ± 3
Capoten (lot #1)	25	23.1–26.9	24.6	24.4 ± 0.7
Capoten (lot #2)	25	23.1–26.9	24.4	24.3 ± 0.5
Zerit	30	28.50–31.50	29.45	29.5 ± 0.7
Zerit	40	38.00–42.00	39.54	41.4 ± 0.9

furthermore for ampoules. For all other ampoules, deviations were within the allowable range.

A number of substances, tablets and capsules have been procured from the US Food and Drug Administration. These substances and dosage forms were accompanied with analysis certificates in which their purity and active ingredient content were specified through conventional drug quantitation methods such as HPLC, titrimetry, and potentiometry. We have analyzed the same samples using organic elemental analysis. The respective comparison of analysis results obtained with conventional methods and with elemental analysis is given in Table 5.

As seen in Table 5, the results obtained with elemental analysis compared to conventional drug quantitation methods were closely spaced, thus providing reliable evidence for the utility of the quantitation method based on elemental analysis.

As a result of our research, it was shown that organic elemental analysis can be used for fast quality control of various pharmaceutical preparations (tablets, capsules, powders, and solutions). It can be concluded that the OEA approach enables fast and accurate determination of active ingredient content and eliminates the need for the use of the reference samples for each active pharmaceutical ingredient.

The benefits of the proposed OEA approach for pharmaceuticals authenticity/quality control are as follows:

- The approach is universal and can be used in standard conditions applicable to all analytes.
- There is no requirement for reference samples or calibration for each individual compound.
- There is no need for extraction.
- As a result of short analysis times, sample throughput is high.
- Complete automation and minimal sample preparation.
- Analysis can be carried out for sample quantities of 1 mg and less, which is important for highly expensive pharmaceuticals.
- In most cases of counterfeit pharmaceuticals, the active ingredient content does not correspond to the specified one; therefore the suggested approach would represent a means to determine these products.

Currently the general article 'Elemental analysis' is being considered for inclusion in Russian Pharmacopoeia by the Russian Ministry for Public Health.

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