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A sequential injection analysis system for potassium clavulanate determination using two potentiometric detectors

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Abstract

Two almost independent potentiometric methods were implemented in a multi-task flow system based on the concept of sequential injection analysis for the analytical control of potassium clavulanate in pharmaceutical formulations. In addition to in-line preparation of the solutions submitted to the analysis, the system simultaneously detected the clavulanate anion and the potassium cation using two potentiometric detectors selective to each of the species referred. For the two determinations, the system developed presented an analytical range of 2 mM–0.1 M. Relative standard deviations of 0.5 and 0.6%, respectively, for clavulanate and potassium, were calculated from the signals obtained for 10 consecutive injections of a solution of 5×10^{-3} M potassium clavulanate. The frequency of sampling was 53 samples h^{-1}. The quality of results supplied was evaluated by comparison with the reference procedure revealing relative errors less than 2% for the two determinations. The simultaneous attainment of two measurements permitted the standardisation of results in real time, the detection of faults in the procedure and monitoring the chemical stability of clavulanate.

Keywords: SIA; Real-time accuracy assessment; In-line calibration; Potassium clavulanate; Potentiometric detection

1. Introduction

The analytical control of pharmaceutical formulations requires the selection of adequate techniques in respect to the quality of results that they produce and the possibility of functioning on a large scale basis. In this sense, automatic methodologies based on continuous flow techniques have been proposed that additionally allow economy of solutions and reduced analysis time [1]. Recently a multi-task flow system with potentiometric detection was proposed which allowed not only the carrying out of the evaluation of the detector units but also the automatic implementa-

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tion of direct potentiometric procedures, the standard addition and titrations, requiring the operator to prepare only the stock solutions of the chemical species involved in the determination [2]. In this work its application in the development of a procedure of accuracy assessment in real time [3] is evaluated. Usually the accuracy assessment of a routine procedure is based on the verification of the quality of results against those obtained by a reference method periodically carried out or by analysis of certified samples. The sequential injection analysis technique (SIA) [4] presents the advantage of permitting the simultaneous accommodation of different types of procedures on the same sample without having to physically reconfigure the system, therefore facilitating the implementation of the accuracy assessment in real time. Taking advantage of this possibility, the development of a procedure for the control of pharmaceutical formulations is proposed, which could be used on a large scale basis with a high analytical performance. Clavulanic acid, *Z*-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, is a potent inhibitor of β-lactamases and is commercialised in pharmaceutical formulations in association with amoxicilin [5]. For its determination in pharmaceutical formulations several spectrophotometric [6–10], fluorimetric [11], polarographic [12] and HPLC methods with spectrophotometric [13–15] or chemiluminescence [16] detection have been proposed. However, the quality of results supplied by these techniques depends on the careful processing of samples, correct maintenance of equipment and periodic verification of results.

The resort to ion-selective electrodes minimises some of the drawbacks mentioned, above all owing to its mode of functioning, selectivity and sensitivity. In this work the potential of the simultaneous use of two electrodes selective to clavulanate [17] and potassium is outlined, in the analytical control of potassium clavulanate in pharmaceutical formulations. These detectors when constructed with a tubular configuration, also present the advantage of being easily incorporated into the system in a robust form, allowing a reduced analytical cost and an elevated sampling rate.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with deionized water (conductivity < 0.1 μS cm^{-1}) and reagents of p.a. or similar quality, without additional purification.

For the preparation of the membranes of the ion selective electrodes (ISE) bis (triphenylphosphoranylidene) ammonium chloride (Aldrich-12403CS, Steinheim, Germany), valinomycin (Fluka-94675, Steinheim, Switzerland), 2-nitrophenyloctyl ether (Fluka-73732, Steinheim, Switzerland), bis(2-ethylhexyl) sebacate (Fluka-84818, Steinheim, Switzerland) *p*-*t*-octylphenol (Fluka-75070, Steinheim, Switzerland), potassium tetrakis(4-chlorophenyl)borate (Fluka-60591, Steinheim, Switzerland) high molecular weight PVC (Fluka-81392, Steinheim, Switzerland) and tetrahydrofuran (Merck-1.09731, Darmstadt, Germany) were used.

The potassium clavulanate (Cipan 370.70H011, Castanheira do Ribatejo, Portugal) and pharmaceutical formulations acquired in local drugstores were stored in darkness under cold, dry conditions.

A 0.033 M ammonium sulphate solution (Riedel-deHaen D-3016, Seelze, Germany) was used as ionic strength adjuster ($I = 0.1$ M and pH 5.6) and as a carrier in the flow system proposed.

The stock solutions of potassium clavulanate 1×10^{-1} and 2×10^{-2} M were prepared immediately before their use, by rigorous weighing out of the solid and dissolution in the ionic strength adjuster solution.

The sample stock solutions were prepared by weighing out a quantity of powder equivalent to 237.3 mg of potassium clavulanate, that resulted from the pulverisation and homogenisation of 20 tablets. Each amount was transferred to a volumetric flask of 2500 ml with 15 ml of water, the mixture being placed under stirring in an ultrasonic bath for 10 min. The volume was adjusted to 2500 ml with water and the solutions were filtered. The potentiometric analyses were carried out in an aliquot of the filtrate that was properly diluted with the ionic strength adjuster in order to obtain a

clavulanate solution with a concentration of about 1×10^{-2} M.

For the analyses carried out by the reference method [18] a volume of the sample stock solution was diluted with water until achievement of a final clavulanate concentration of about 9.8×10^{-4} M.

2.2. Construction of electrodes

The tubular electrodes, selective for clavulanate and potassium, with PVC membrane and without internal reference solution, were constructed according to the procedures previously described [19], depositing the sensory membrane in the conductor surface of the central electrode hole.

The membrane used in the construction of the clavulanate electrode [17] was prepared by a mixture of 1.3% (w/w) bis (triphenylphosphoranylidene) clavulanate, 64.5% (w/w) 2-nitrophenyloctyl ether, 3.2% (w/w) *p-t*-octylphenol and 31.0% (w/w) PVC. For the potassium electrode the membrane preparation was followed mixing 1.0% (w/w) of valinomycin, 65.8% (w/w) bis(2-ethylhexyl) sebacate, 0.2% (w/w) potassium tetrakis(4-chlorophenyl)borate and 33.0% (w/w) of PVC. The membranes were left to dryness and conditioned in a 10^{-2} M solution of primary ion for 12 h.

2.3. Equipment and software

The multi-task flow-system (Fig. 1), whose mode of functioning was previously described [2] was made up of a peristaltic pump Gilson Minipuls 3 (Villiers-le-Bel, France), equipped with a PVC propulsion tube, of the same brand, with a 1.85 mm internal diameter. The other components included a six-port rotatory valve (RV) from Valco Instruments, model Cheminert C15-3186E (Houston, USA); a three-way solenoid valve (SV) NResearch 161 T031 (Stow, USA); two potentiometers Crison micropH-2002, sensitivity ± 0.1 mV (Barcelona, Spain) that measure the difference of potential between the indicator electrodes and a reference electrode of double junction AgCl/Ag model 90-0029 from Russel (Fife, Scotland), containing a 0.033 M ammonium sulphate solution in the external compartment.

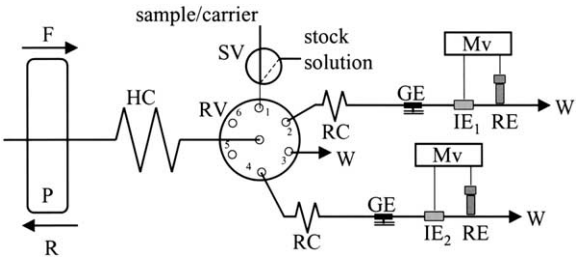

Fig. 1. SIA system for potassium clavulanate determination in pharmaceuticals. P = peristaltic pump; RV = rotatory valve; SV = three way solenoid valve; GE = grounding electrode; IE_1 = clavulanate-selective electrode; IE_2 = potassium-selective electrode; RE = reference electrode; Mv = decimilivoltimeter; W = waste.

The manifold was constructed with PTFE tubing of 0.8 mm internal diameter; the serpentine holding coil (HC) was 400 cm in length, and the reaction coils (RC) were 40 cm each. Supports were used for the tubular and reference electrodes and the grounding electrodes were constructed in the laboratory [20].

The system was controlled by a computer through an Advantech PCL 711B interface card with the system control and signal acquisition programme having been developed in QuickBasic language.

The sample analysis by the method proposed by the United States Pharmacopoeia [18] was carried out in a chromatographic Merck Hitachi system (Tokyo, Japan), made up of a pump (model 7100), connected to an Rheodyne injector model 7725i (loop of 20 μl) and a silica column Lichrocart RP 18 (250 × 4 mm), packed with Lichrosorb 5 μm particles. The detector used was a diode array system, model 7000, with the data processed through software of the same brand (Model D7000).

2.4. Procedures

Initially the HC and transmission channels between the RV and detection systems were filled with carrier/adjuster of ionic strength solution selecting ports 2 and 4 and placing the peristaltic pump in propulsion mode. This mode of operation was maintained until a stable baseline was obtained for the electrodes. The instructions required for an analytical cycle for determination of the

clavulanate anion and of the potassium cation are defined in Table 1. The first three steps of the calibration procedure and first two steps of the measurement procedure correspond to the substitution of the solutions that fill the system.

The selection of port 1 (first step of the calibration cycle) with the SV activated enabled the filling up of the active access channel with a 2×10^{-2} M solution of potassium clavulanate for the evaluation of the slope of the electrode selective to clavulanate or the electrode selective to potassium. When the SV was not activated the passive channel was filled with carrier solution for the calibration procedure of the potentiometric detectors or with sample solution in the case of analytical determinations.

The determination of the electrode slopes was carried out by four times selection of the two last steps of the calibration cycle, involving the RV ports 1 and 2 in the case of clavulanate ISE and 1 and 4 in the case of potassium ISE. Potassium clavulanate calibration solutions with respective concentrations of 5×10^{-3}, 1×10^{-2}, 1.5×10^{-2} and 2×10^{-2} M were automatically prepared in the interior of the system for each selection of port 1, by means of the SV on/off cycles. Small plugs of the standard of potassium clavulanate (2×10^{-2} M) were aspirated into the HC by activation of the SV (SVon) in alternated mode, with plugs of carrier solution, when the SV was in the off position (SVoff). The mutual dispersion between the two solutions inserted during the stage of

Table 1
System operation

RV	SV state[b]		Volume (μl)	Flow-rate (ml min^{-1})	Flow direction	Event
Clavulanate or potassium[a] *selective electrode calibration*						
1	ON		200	1.5	R	Stock potassium clavulanate solution
1	OFF		200	1.5	R	Carrier
3	–		600	4.0	F	Waste
1	ON	25% 50% 75% 100%	375	1.5	R	In-line clavulanate standard preparation
2	–		4000	2.0	F	Clavulanate measurement
or[a]						
1	ON	25% 50% 75% 100%	500	9.8	R	In-line potassium standard preparation
4	–		41 700	5.0	F	Potassium measurement
Clavulanate or potassium[a] determination						
1	OFF		200	1.5	R	New sample
3	–		400	4.0	F	waste
1	OFF		375	1.5	R	Clavulanate sample volume
2	–		4000	4.0	F	Clavulanate measurement
or[a]						
1	OFF		500	9.8	R	Potassium sample volume
4	–		4170	5.0	F	Potassium measurement

Steps for potassium clavulanate determination in pharmaceutical formulations with the SIA system in Fig. 1. R = reverse; F = forword; RV = rotatory valve; SV = solenoide valve.

[a] Potassium procedures.

[b] Each SV on/off cycle has 2 s.

aspiration into the HC and during the sending to the detector permitted simulation of the intercalation of the solutions with the mentioned above concentrations. To obtain the four standards the SV was activated during 25, 50, 75, and 100% of the time for each on/off cycle applied to the SV.

For the calibration of the clavulanate ISE, 375 μl of solution (corresponding to eight cycles) were aspirated and sent to the detector at a flow rate of 2.0 ml min^{-1}. For the analytical determinations, port 1 was selected and filled with sample solution to be measured. A sample volume equal to that mentioned above was aspirated into the HC and sent to the detector (port 2) with a similar flow rate. For the calibration of the potassium ISE, 500 μl of solution was aspired in port 1 (corresponding to five cycles) and sent to the detector (port 4) at a flow rate of 5.0 ml min^{-1}. The measurement was carried out in a mode similar to that of clavulanate, aspiring to the HC, through port 1, a sample volume of 500 μl that was sent to the detector at a flow rate of 5 ml min^{-1}.

3. Results and discussion

3.1. Optimisation of the system proposed

As is the characteristic of continuous flow methodologies, the injection of reduced volumes of sample, in a liquid flow stream, determines its physical dispersion leading to a transient analytical signal in the detector, of reduced intensity when compared with that obtained by conventional analysis using an increased sample volume. In these circumstances, the reproducibility of measurements depends on the constancy of the hydrodynamic variables of the system, verified periodically in a direct form or by carrying out calibration procedures. The minimisation of physical dispersion of the solutions injected can contribute to the increase in robustness of analysis in that the results are less influenced by small changes of the system working conditions. With the objective of guaranteeing a degree of robustness to the proposed procedures, the intensity and repeatability of the potentiometric signals obtained was studied either by varying the injection volume for the two determinations in the interval 150–1000 μl and the propulsion flow in the interval 2–8 ml min^{-1} in a way whereby the sampling rate was not diminished. In these experiments, the RC was fixed initially at 20 cm with which it would be possible to minimise the volumes of solutions spent per determination. It was verified however that the proximity of the RV detector devices introduced an electrical noise in the measurements, difficult to eliminate with the grounding electrode inserted in line, before the detection system. The RC length was therefore increased to 40 cm. With the increase of injection volume, an increase in the intensity of analytical signals was verified up to volumes of 375 and 500 μl, respectively, for clavulanate and potassium, as a consequence of decreasing the dispersion effect. For greater injection volumes no significant improvement in the intensity of signals was observed. These injection volumes were obtained for maximum flow rate of 2 and 5 ml min^{-1}, respectively, for clavulanate and potassium. The increments of the flow rate carried an appreciable loss of signal repeatability, possibly caused by inertia to the flow during the aspiration stage and by the dynamic response characteristics of each of the electrode types used.

As previously demonstrated [2], the system enabled in-line preparation of solutions using the SV valve housed in port 1. By means of successive momentary activations of SV, during aspiration, through port 1 it was possible to create a stack of small zones of stock solution of potassium clavulanate and of ionic strength adjuster solution. The aspiration and inversion of flow direction contributed to the homogenisation of potassium clavulanate concentration in all segments, with the final concentration being determined by the proportion of time during which the SV remained activated. By fixing the on/off cycle time at 2 s, it was possible to guarantee a sufficiently reduced volume of the segments to permit an effective homogenisation, even when the SV remained activated during 1% of each cycle. This time was sufficient to allow a reproducible aspiration of the two solutions. The longer aspiration of solutions from port 1, with the application of an increasing number of cycles, illustrated the attainment of

Table 2
Determination of clavulanic acid pharmaceuticals formulations (mg clavulanic acid/tablet)

Samples	Reference method[a]	ESI clavulanate[a]	R.D. (%)[b]	ESI potassium[a]	R.D. (%)[b]
Augmentin	136.8±0.6	134.5±1.6	−1.7	136.6±1.2	−0.1
Betamox	143.9±0.5	142.1±2.0	−1.3	143.1±1.7	−0.6
Clavamox	125.2±1.6	126.3±1.3	0.9	125.8±1.4	0.5
Penilan	113.3±0.6	115.6±1.3	2.0	111.3±1.4	−1.8

[a] Average of four determinations±standard deviation.
[b] Relative deviation to the reference method.

analytical signals for the determination of clavulanate and potassium with increasing intensity. Analytical signals were obtained with an intensity of 95% when compared to the intensity obtained for the conventional analysis of the same calibration solutions when 375 μl (eight cycles) were aspirated for the clavulanate detector and 500 μl (five cycles) for the potassium detector. Equally, the calibration carried out with the 5×10^{-3}, 1×10^{-2}, 1.5×10^{-2} and 2×10^{-2} M standards of potassium clavulanate, prepared in the interior of the system, were compared with those prepared manually and no differences in the intensity of analytical signals obtained have been observed.

Having optimised the flow conditions regarding the flow rate and injection volumes, the characterisation of ISEs was then proceeded with. It was shown that the unit selective to clavulanate presented a Nernst response in the concentration interval of 2×10^{-3}–1×10^{-1} M, with a slope of -60.5 ± 0.5 mV decade^{-1}. A relative standard deviation of 0.6% was obtained for 10 consecutive injections of standard 5×10^{-3} M potassium clavulanate solution, with the sampling frequency being 55 injections h^{-1}. The potassium ISE exhibited a linear response in the interval 2×10^{-3}–1×10^{-1} M, with a slope of 56.6 ± 0.2 mV decade^{-1}. Following 10 consecutive injections of standard 5×10^{-3} M potassium clavulanate solution, it was observed that the rsd of the results was 0.5%, with the sampling frequency being 53 injections h^{-1}.

3.2. Analytical applications

Having optimised the flow conditions and defined the characteristics of the ISEs, a determination of clavulanic acid in pharmaceutical formulations commercialised in Portugal was proceeded with, by direct potentiometry following the procedure referred to in Table 1. To evaluate the quality of the potentiometric results obtained, a determination of clavulanic acid on the same samples resorting to the reference method proposed by the USP [18] was carried out in parallel. The results obtained are shown in Table 2. The application of the student t-test for the pairs of values obtained, showed an absence of statistical differences for a confidence level of 95%. The

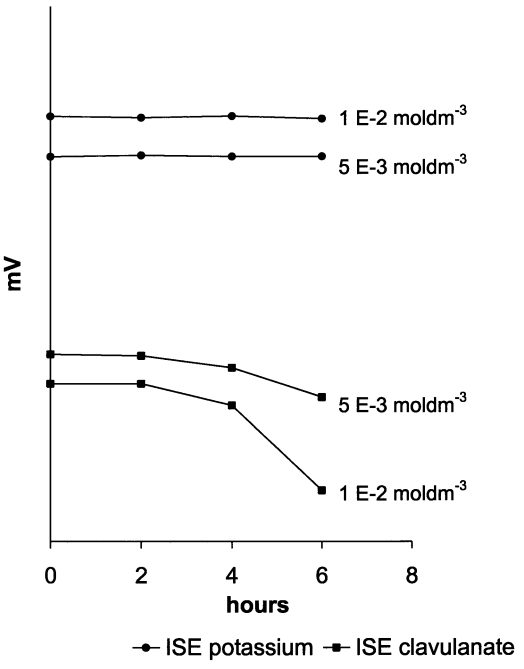

Fig. 2. Effect on signal intensity by exposure a potassium clavulanate solution (5×10^{-3} and 1×10^{-2} mol dm^{-3}) to air and light.

exposure of samples to the air and light leads to a rapid degradation of clavulanate (Fig. 2) and gives rise to a progressive deviation between the measurements obtained with the proposed system. However, the mean of the results obtained by the two procedures could be more accurate. The random errors are minimised and the nature of the systematic errors arising from the presence of interfering species is different for the two ISEs. The analysis of the relative errors or the absence of agreement of results could therefore indicate a fault of one of the procedures and the consequent necessity of system verification, or the degradation of clavulanate in the pharmaceutical formulations.

4. Conclusions

The possibility of coupling in a robust form, two potentiometric detectors, sensitive to anion and to cation, of simple construction, to a SIA system permitted the evaluation of potassium clavulanate in pharmaceutical formulations by two different methodologies. The system developed was easy to operate, gave rise to a reduced consumption of reagents, favoured an elevated sampling frequency, an accuracy of results estimated in real time and proved useful in analytical control of pharmaceutical formulations.

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