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Flow-injection determination of phenylephrine hydrochloride in pharmaceutical dosage forms with on-line solid-phase extraction and spectrophotometric detection☆

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Abstract

A flow injection method is proposed for the determination of phenylephrine hydrochloride in pharmaceutical dosage forms. The method involves the use of on-line solid-phase extraction by means of a microcolumn containing Dowex 50W X8 ion-exchange resin for the separation of the analyte prior to colour development and spectrophotometric detection in the visible region.

The influence of preconcentration flow, preconcentration pH and elution volume was studied.

The method exhibits appropriate linearity ($r^2 = 0.9999$) which was proved statistically by means of the "*F*"-test. When applied to commercial samples containing several active ingredients and excipients, a significant reduction of interferences was found. Accuracy, evaluated by means of the spike recovery method was in the range 99.7–100.8%, with precision (R.S.D., %) better than 1%.

In order to achieve the automation the system was controlled from a notebook computer by means of a program written in QuickBASIC language. Under these conditions, a sampling frequency of 40 samples per hour could be attained.
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Keywords: Flow injection; Pharmaceutical; Phenylephrine; Solid phase extraction; Spectrophotometric detection

1. Introduction

Phenylephrine hydrochloride ((R)-3-hydroxy-α-[(methylamino)methyl] benzenemethanol hydrochloride) is a sympathomimetic drug used for nasal congestion, sinusitis and rhinitis. It is also used in ophthalmology as a mydriatic and conjunctival decongestant [1]. It is incorporated in a number of pharmaceutical preparations either alone or, more frequently, associated with other active ingredients. Dosage forms include tablets, syrups, eye drops and injections.

Among the methods currently established for the determination of this substance, one of the most frequently used is high performance liquid chromatography (HPLC), for instance in the United States Pharmacopeia (USP) for the determination in pharmaceutical dosage forms (Phenylephrine Hydrochloride Injection, Nasal Jelly and Ophtalmic Solution) [2]. The same is true for the British Pharmacopoeia [3] in the Phenylephrine Eye Drops monograph.

Direct spectrophotometric measurements can be carried out only in the absence of other UV-absorbing substances. An example of this is the Phenylephrine Injection monograph in the British Pharmacopoeia. Visible spectrophotometry [4] and chemiluminescence [5] have also been proposed. Unfortunately, chromogenic or luminogenic reactions may be interfered by excipients or other active ingredients co-formulated in the dosage form.

The literature is scarce in methods for the determination of phenylephrine in more complex dosage forms, consisting of several active substances or more complicated vehicles, which are often found in oral solutions. Spectrofluorimetry has been proposed for this purpose [6,7]. HPLC is also a good technique for resolving complex mixtures, however, the investment and operating costs are high. For some purposes, the high investment may not be justified.

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Thus there is the need for selective methods capable of affording this determination especially for routine quality control.

The separation of phenylephrine from the interfering substances can be attained in a simple and inexpensive way by means of solid phase extraction. Solid phase extraction (SPE) [8,9] has been extensively used as a sample preparation tool together with different detection techniques, either as a cleanup tool or for separating the analyte for later elution. In fact, one of its earliest forms, ion exchange, has been used for a long time for the separation of some species of pharmaceutical interest, among them phenylephrine itself [10].

Besides being rather slow, one of the drawbacks of the application of SPE in the classical batch form, is the risk of losses or contamination associated to multi-stage procedures such as those involving colorimetric determination. On the other hand, when SPE is incorporated in a flow-injection analysis (FIA) [11–13] system, the power of the combined technique is increased and the mentioned risks are virtually eliminated. Furthermore, the accurate timing inherent to FIA eliminates the need for lengthy waits for colour developments.

However, the application of the concept of SPE-FIA to the pharmaceutical and related fields is not extensive yet, and few papers have been found in the literature [14–16].

The aim of this work was to design an automated system for the determination of phenylephrine hydrochloride in pharmaceutical dosage forms without interference from coformulated substances. For this purpose, a flow-injection system involving on-line solid-phase extraction with spectrophotometric detection in the visible region was developed and assessed. The detection was carried out exploiting the Emerson reaction with 4-aminoantipyrine and potassium hexacyanoferrate(III). This is a well-known reaction of phenolic substances [17–21], already proposed by Hiskey and Levin [4] for the determination of phenylephrine in batch form.

2. Experimental

2.1. Manifold

The flow injection system is depicted in Fig. 1. A Gilson (Villiers-le-Bel, France) Minipuls 2 multichannel peristaltic pump fitted with Tygon pump tubing was used as main pump. Commutations were performed by means of three Valco (Houston, USA) Cheminert 6-port injection valves. Valves V2 and V3 were fitted with loops, 750 μL and 50 μL, respectively.

The column (diameter 4 mm, length 26 mm) was constructed drilling a cylindrical hole in a piece of acrylic material. In order to retain the resin in place, the acrylic block was capped by means of two small pieces of stainless steel mesh, retained by acrylic caps and screws. The seal was attained by means of a thin silicone rubber layer at each of the extremes between the block and the caps.

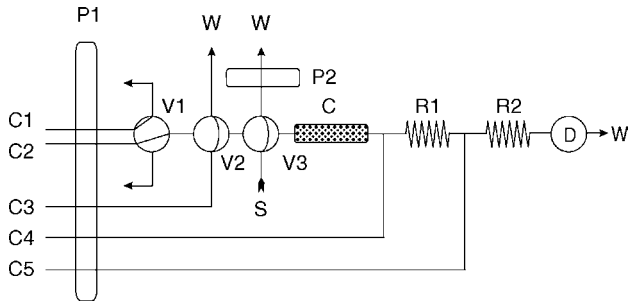

Fig. 1. Diagram of the SPE-FIA system. P1: main peristaltic pump; P2: auxiliary peristaltic pump; C1: phosphate buffer 0.01 M pH 5.9, 1.02 mL min^{-1}; C2: water, 1.0 mL min^{-1}; C3: 0.1 M sodium hydroxide, 3.9 mL min^{-1}; C4: 4-aminoantipyrine 0.4% in water, 0.78 mL min^{-1}; C5: potassium hexacyanoferrate(III) 2% in 1% sodium carbonate, 0.78 mL min^{-1}; S: sample, 2.2 mL min^{-1}; R1: reactor, 100 cm; R2: reactor, 200 cm; V1, V2, V3: 6-port injection valve, Valco Cheminert, V2 with 750-μL loop, V3 with 50-μL loop; D: detector (spectrophotometer at 500 nm); W: wast.

The signal was detected and recorded in a Shimadzu (Kyoto, Japan) UV-240 recording spectrophotometer operated at 500 nm in the time-scan mode and fitted with a Hellma (Müllheim, Germany) quartz flow cell with a 10-mm optical path (internal volume 80 μL). The signal was recorded on the chart recorder of the instrument and peak-height measurements were obtained by means of the "Peak-pick" function of the instrument.

Some experiments were also carried out in a Hewlett-Packard HP-8453 diode-array spectrophotometer fitted with the same 80-μL cell. It was found that the absorbance values obtained under similar conditions were higher with this instrument. This could be explained by the difference in geometry of the radiation beam passing through the flow cell in this instrument, which is of the reverse-optics type. Otherwise results from both instruments were comparable. Signals reported in Fig. 2 were obtained with the Hewlett-Packard instrument.

Connections and reactors were made with 0.8-mm (internal diameter) Teflon FEP tubing. Valco Cheminert flangeless fittings were used throughout.

2.2. Operation of the system

Valve V1 is configured to select the carrier, either C1 (buffer) or C2 (water). Buffer is the preconcentration medium while water is used for rinsing the column and eliminating the buffer remaining within the microcolumn so that it does not interfere with the sodium hydroxide solution during the elution step. The carrier not selected at a given time is recirculated to the bottle by valve V1.

In the beginning of an analytical cycle, V1 is set to select buffer for 10 s for conditioning the column. Simultaneously, the sample is loaded into the loop of valve V3 by means of an auxiliary peristaltic pump (P2) and sodium hydroxide solution (C3) is loaded into the loop of valve V2. Valve V3

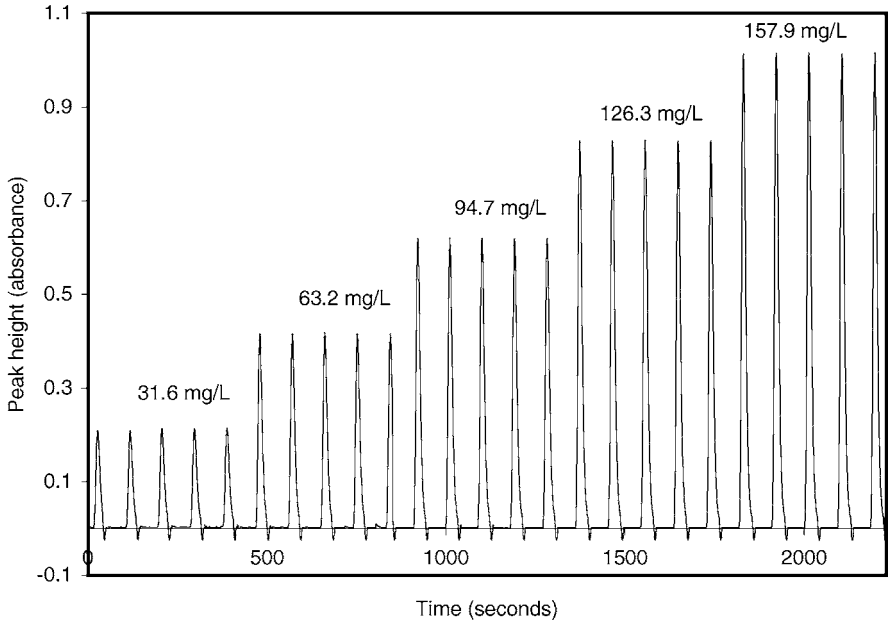

Fig. 2. SPE-FIA signal trace of a phenylephrine hydrochloride calibration curve. Signals obtained with a Hewlett-Packard HP-8453 spectrophotometer fitted with a Hellma 10 mm × 10 mm 80-μL flow cell.

is then rotated and the sample injected in the buffer stream and carried to the microcolumn C where phenylephrine is preconcentrated for 5 s. Then V1 is switched and water is circulated for 10 s for rinsing the column. After this period, V2 is switched and sodium hydroxide solution is injected into the water stream. Phenylephrine is eluted and mixed with the rest of the chromogenic reagents in reactors R1 and R2. Signals can be seen in Fig. 2.

2.3. Automation

Automation of the process was achieved by means of a program written in QuickBASIC version 4.0 (Microsoft) and running in a personal computer under MS-DOS 5.

The three Valco valves are fitted with microelectric actuators and can receive commands via the serial (RS-232) port of the PC. The firmware in these valves allows to configure each valve with a specific address (0–9) thus all three valves can be connected to the same RS-232 port and commanded individually by addressing the commands to the specific valve.

The auxiliary pump was switched on and off from the PC via the LPT1 parallel port and an optocoupled triac interface capable of switching the 220 V mains voltage.

2.4. Reagents

Dowex 50W X2 and X8 ion exchange resin (50–100 mesh) was obtained from Fluka (Buchs, Switzerland). BAKERBOND spe® sorbent (aromatic sulfonic acid, carboxylic acid) was obtained from dismantled BAKERBOND (J.T. Baker, Phillipsburg, USA) columns. L-phenylephrine hydrochloride (Sigma, St. Louis, MO, USA, declared assay 100%) was used as received. 4-Aminoantipyrine was obtained from Fluka. The rest of the reagents were of analytical reagent grade.

2.5. Solutions

Sodium carbonate 1% (m/v) in water.

4-Aminoantipyrine 0.4 % (m/v) in water.

Potassium hexacyanoferrate(III) 2% (m/v) in 1% sodium carbonate.

Phosphate buffers were prepared from potassium dihydrogen phosphate and disodium hydrogen phosphate at final concentration 0.01 mol L^{-1} and adjusted to final pH values of 8.1, 6.9, 5.9 and 4.9.

All reagent solutions were filtered through 0.45-μm membrane filter.

Standard solutions were prepared fresh daily dissolving phenylephrine hydrochloride in water and diluting to the desired concentration with the same solvent.

2.6. Commercial samples

Rondec NF (Abbott, Uruguay) syrup containing: phenylephrine hydrochloride 2 mg mL^{-1}, caffeine 7 mg mL^{-1}, carbinoxamine maleate 0.8 mg mL^{-1}.

Couldina (Alter, Spain) effervescent tablets containing each phenylephrine hydrochloride 7.5 mg, acetylsalicylic acid 500 mg, chlorpheniramine maleate 2 mg.

2.7. Calibration

Five-point calibration curves in the range 30–160 mg L^{-1} phenylephrine hydrochloride were fitted to a linear model by means of the least-squares method.

2.8. Sample preparation

Syrup samples were diluted with water as needed. Effervescent tablets (10 U) were treated in a 500-mL volumetric flask with 100 mL of water until effervescence ceased. Methanol (300 mL) was added and the flask treated in an ultrasonic bath for 10 min, then made up to volume with the same solvent and filtered through a 0.45-μm PTFE membrane filter. For the determination, 25 mL of the solution were diluted with water to 50 mL. For the spike addition method, 25 mL of the solution were spiked with the appropriate amount of standard solution and diluted to 50 mL with water.

3. Results and discussion

3.1. Selection of the sorbent

Given the chemical nature of the analyte, it is reasonable to expect that under the cationic (ammonium) form, it will be retained by an anionic sorbent. Several types of sorbents were tested, namely BAKERBOND spe® sulfonic and carboxylic packings (consisting of silica gel chemically modified, respectively with sulfonic acid and carboxylic acid groups), as well as ion-exchange resin Dowex 50W (sulfonic acid groups), the latter with two different crosslinking grades, X2 and X8. The Bakerbond packing with carboxylic groups was totally unsuitable because quantitative retention could not be achieved. The Bakerbond packing with sulfonic groups attained good results in terms of recovery and short-term precision, however, the chemical resistance of the column to repeated injection of the eluent (sodium hydroxide solution) was poor. The Dowex 50W X2 was chemically stable, but turned out to be unsuitable due to the high shrinking and swelling properties exhibited. Thus, the Dowex 50W X8 resin was finally chosen for packing the microcolumn.

3.2. Eluent

Phenylephrine is effectively eluted from the resin at high pH values. Fortunately this is compatible with the colour development reaction, which is carried out in alkaline medium. Therefore, sodium hydroxide solution was chosen as eluent. In order to minimise refraction-index effects, the lowest possible concentration was chosen. Sodium hydroxide concentrations of 0.01 M and 0.1 M were tested. With the former, quantitative elution was not possible with loops of reasonable length thus 0.1 M was selected.

The amount of sodium hydroxide solution necessary to ensure a complete elution was studied by varying the size of the loop in valve V2. Two sizes were tested, 500 μL and 750 μL. Complete elution with 1 loop volume was possible with 750 μL but not with 500 μL, thus the higher volume was finally selected.

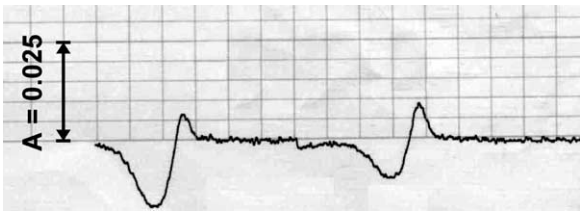

Fig. 3. Plot of the signal corresponding to two blank injections, showing residual Schlieren effect.

3.3. Effect of refraction-index change

One of the problems associated with on-line preconcentration with optical absorptiometric detection is the spurious signal observed during elution due to the variation of refraction index of the fluid when the eluent reaches the flow cell (an effect referred to as “Schlieren effect”). This effect is usually more noticeable in the UV region and can hinder the determination if the absorptivity of the analyte is low, as happens with phenylephrine. Hence colour development prior to detection was chosen in order to enhance the absorbance.

With the current system the signals caused by the Schlieren effect were negligible with the sodium hydroxide concentration of 0.1 M. Only a small negative peak can be seen at the end of each peak, as depicted in Figs. 2 and 3.

3.4. Influence of operating conditions

The influence of preconcentration flow rate and pH was studied by means of univariant experiments. For the study of the influence of flow rate, preconcentration was carried out using flow rate values of 0.78, 1.02, 1.28, 1.51 and 1.86 mL min^{-1} using water as carrier and maintaining other variables constant. For the study of the influence of pH, buffers of pH values of 8.1, 6.9, 5.9 and 4.9 were used as carriers while maintaining the preconcentration flow rate fixed at 1.02 mL min^{-1}. For each value of the variable, five-point calibration curves in the range 30–160 mg L^{-1} of phenylephrine hydrochloride were measured in triplicate. Regression equations (linear fit with intercept) and regression variance (s_R^2) were calculated.

The rationale for this experiments is as follows. For a flow method based on column preconcentration, linearity of the calibration curve and sensitivity (slope of the calibration curve) are dependent on the operating conditions. When these conditions are inappropriate a reduction of the linear range (increased curvature of the calibration curve) and a smaller regression slope may be observed due to the reduced capacity of the sorbent for retaining higher analyte masses, and the method then becomes non-quantitative due to column breakthrough. Hence, regression slope (sensitivity) and regression variance (fit) can be taken as indicators for the overall capacity of the column for retaining the analyte. In

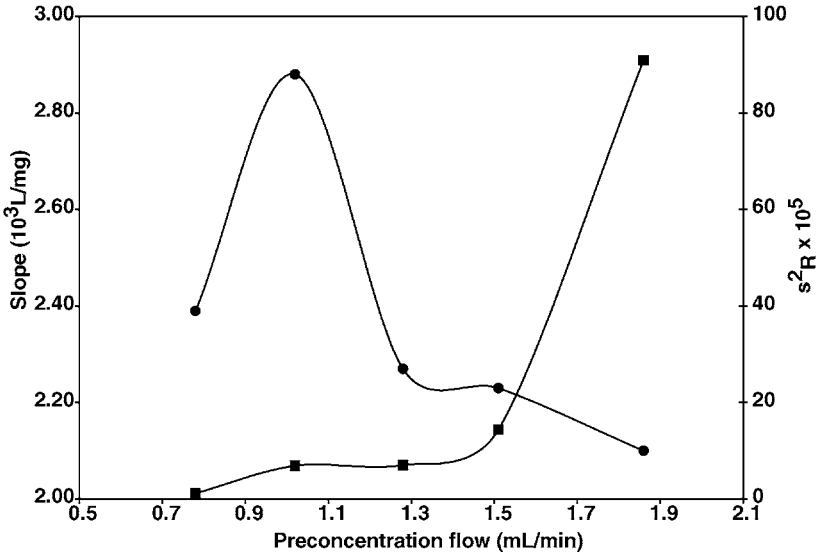

Fig. 4. Effect of preconcentration flow on slope (●) and regression variance (■) of the calibration curve (regression model $h = aC + b$). Carrier: water; elution: NaOH 0.1 M, 750 μL.

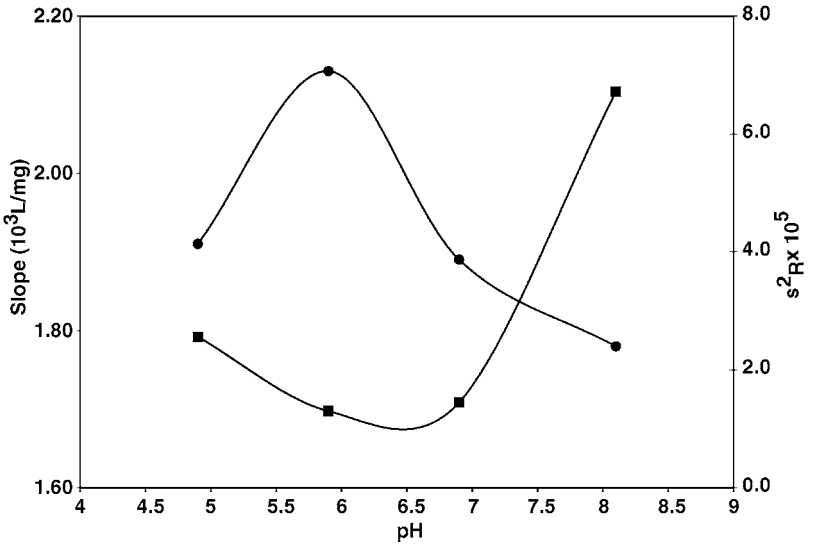

Fig. 5. Effect of preconcentration pH on slope (●) and regression variance (■) of the calibration curve (regression model $h = aC + b$). Carrier: phosphate buffer of corresponding pH, 1.0 mL min^{-1}; elution: NaOH 0.1 M, 750 μL.

order to ensure that the retention would be quantitative, the experiment was designed with concentrations spanning a range up to 200% of the analytical concentration.

Results are presented in Figs. 4 and 5. For each of the two variables those conditions ensuring the highest regression slope and reasonably good linear fit (low regression variance value) were chosen. The best conditions corresponded to a preconcentration flow of 1.02 mL min^{-1} and pH 5.9, hence these values were chosen as standard.

3.5. Sampling frequency

With the time intervals selected and already described the sampling frequency of the system was 40 samples per hour.

4. Validation

Linearity, limit of quantification (LOQ), precision and accuracy were the figures of merit of the method studied in this work.

4.1. Limit of quantification (LOQ)

From experimental data, the LOQ (10σ) was calculated to be 5.8 mg L^{-1} of phenylephrine hydrochloride.

4.2. Linearity

Linearity was investigated by means of a five-point calibration curve in the range up to 160 mg L^{-1} of phenyle-

Table 1
Results from the recovery assays performed on spiked and unspiked commercial samples

Sample, label claim	Unspiked content found/precision (R.S.D., %)		Recovery (%) at spike level 50%		Recovery (%) at spike level 100%	
	Direct FIA	SPE-FIA	Direct FIA	SPE-FIA	Direct FIA	SPE-FIA
Rondec NF ($2\,mg\,mL^{-1}$)	$2.18\,mg\,mL^{-1}$ (0.53)	$1.99\,mg\,mL^{-1}$ (1.19)	108.6	99.7	107.8	100.8
Couldina (7.5 mg per tablet)	8.13 mg per tablet (0.43)	7.67 mg per tablet (0.41)	104.2	100.2	103.5	100.0

Direct FIA: results obtained without SPE separation; SPE-FIA: results obtained with SPE separation. For other details see text.

phrine hydrochloride, which spans twice the analytical concentration. Each solution level was injected five times. The regression equation fitted a linear model in the range 5.8–160 $mg\,L^{-1}$: $h = 0.00222C + 0.0204$ ($r^2 = 0.9999$), h being peak height (absorbance) and C the concentration of phenylephrine hydrochloride ($mg\,L^{-1}$). The validity of this model was tested by means of the LOF (lack-of-fit) test. The calculated value for the "F"-statistic was 2.08, lower than $F(3,20,\ 0.05) = 3.10$, hence the linear model can be accepted at the confidence level $\alpha = 0.05$.

4.3. Accuracy and precision

Two different commercial samples available in the local market (oral solution and effervescent tablets) were used to assess the suitability of the method for the determination in real samples. No pharmacopoeial method is available for the determination of phenylephrine in these commercial preparations, and their complete formulations are proprietary, hence the accuracy was assessed by the spike recovery method. After the initial sample treatment (see Section 2.8), spikes corresponding to 50% and 100% of the nominal concentration of phenylephrine hydrochloride were added. The concentrations of phenylephrine hydrochloride were then determined by the proposed method, and percent recoveries calculated. An unspiked sample solution was also analysed. All determinations were carried out in duplicate.

In preliminary experiments, it was found that direct determination of phenylephrine hydrochloride without prior separation of the analyte suffered from interferences in complex formulations such as those analysed in the current work. This was assessed employing the FIA system without the microcolumn and using water as carrier. With the commercial samples (Rondec NF syrup and Couldina tablets) spike recoveries significantly higher than 100% were found suggesting the presence of a multiplicative interference (see Table 1 under "Direct FIA"). However, when employing the SPE-FIA method spike recoveries close to 100% were found, with a precision (relative standard deviation, $n = 5$) of around 1% or better, as shown in Table 1. This precision is surprising given the relative complexity of the system.

5. Conclusions

Flow injection analysis with on-line separation by solid phase extraction provides a useful tool for the determination of phenylephrine in complex pharmaceutical dosage forms. The on-line separation together with the chromogenic reaction provides increased selectivity for this purpose. Linearity, accuracy and precision are appropriate for quality control of pharmaceutical products. The method exhibits a high sampling frequency and is amenable to automation.

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