AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

A Quick & Easy Method for the Determination of Percent Spike Recovery in Pharmaceutical Analyses

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ABSTRACT

The aim of this study is to present a Quick and Easy formula for the calculations of percent spike recovery of drug samples. Currently there are several cumbersome methods used in the determination of percent spike recovery in pharmaceutical analyses performed in various laboratories including the FDA laboratories around the world and especially in USA. In this paper we have compared the Quick & Easy Method using Pal's formula against the method suggested by ORA Manual. The efficacy of this new formula has been demonstrated by seven examples including the main example of Pilocarpine hydrochloride discussed in detail. This Quick & Easy method is very simple, efficient and convenient way of determining percent spike recoveries and RPD values of duplicate spike solutions in drug analyses by HPLC, GC or UV methods. The simplicity of the method saves time and effort when compared to the existing method

Keywords: Pal's formula, ORA Manual

Disclaimer: "The article reflects the views of the authors and should not be construed to represent FDA'S views or Policies"

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Received 20 September 2012, Accepted 06 November 2012

Please cite this article in press as Pal M. *et al.*, A Quick & Easy Method for the Determination of Percent Spike Recovery in Pharmaceutical Analyses. American Journal of PharmTech Research 2012.

INTRODUCTION

Two most important criteria in the validation of any analytical methods are: 1. Linearity 2. Percent Spike Recovery. Generally, linearity is determined once to establish the dynamic range to bracket the lower and upper limit of the analyte in question. Percent recovery is determined routinely as an additional step to ensure the robustness of the method. Additionally, it is a requirement as an integral part of the analysis protocol or Standard Operating Procedure (SOP) of this Laboratory which is in accord with Laboratory Procedure Manual (LPM) and consistent with ISO 17025 mandates. It is also a Current Good Laboratory Practice (CGLP) to calculate recoveries from two separate spike preparations. The resulting two numbers are analyzed statistically to obtain the Relative Percent Difference (RPD), which is an indicator or measure of precision of the analysis. Percent spike recovery varies from analyst to analyst and also depends on the analyte and the matrix. Usually, the matrix effect is very minimal in pharmaceutical analyses unlike the food and pesticide matrices. Hence, the RPD margin in drug spike recovery experiments is always found to be very small.

Currently there are several cumbersome methods used in the determination of percent spike recovery in pharmaceutical analyses performed in various laboratories including the FDA laboratories around the country. In this paper we have compared the Quick & Easy Method using Pal's formula against the method suggested by ORA Manual.

ORA MANUAL METHOD [1]

The % Recovery according to the above method is obtained using the formula:

$$\% \text{ Recovery} = \frac{X\,(100)}{K} \qquad (1)$$

Where, X, an observed value = $\dfrac{(A_{U\,Sam})\ \mathbf{x}\ (\text{Standard weight})\ \mathbf{x}\ (\text{Sample volume})}{(A_{S\,Std})\ (\text{Standard volume})\ (\text{Sample weight})}$

All the three terms used in obtaining a value for X in the above equation are generated during the assay of a drug or analyte in question and therefore experimental details are kept to a minimum. The terms A_U and A_S are responses in peak areas, a US Pharmacopeia notation for unknowns and standards, if the assay was done using High Performance Liquid Chromatography or Gas Chromatography (HPLC, GC) or by absorbance values, if the analysis is performed using UV spectrometry.

The term K, is the total amount of the sample and standard in the spike solution, which is = (Weight of the sample +Weight of the standard). K, the denominator is the same in the two spike recovery calculations.

MATERIALS AND METHOD

Weight of standard = 10.77 mg (***Pilocarpine hydrochloride***)

Purity of standard = 0.998 (99.8%)

Volume of standard = 25.0 mL x 25.0 mL /3.0 mL = 208.33 mL

The standard and sample solutions were prepared using MilliQ water.

Standard preparation

Stock solution

10.77 mg of the standard was dissolved in about 5 mL of water in a 25.0 mL volumetric flask using a mechanical shaker and further diluting with water to the mark. Working standard solution was prepared by diluting 3.0 mL of the stock solution to 25.0 mL in volumetric flask.

Sample preparation

Stock solution

Ten tablets were dissolved in 400 mL water in a 500.0 mL volumetric flask using a mechanical shaker, and making the volume to the mark. The working sample solution was prepared by diluting 5.0 mL of the stock solution to 10.0 mL in a volumetric flask.

Spike solutions

Two separate 1: 1 mixture of solutions: 1.0 mL working standard solution + 1.0 mL working sample solution were prepared.

HPLC conditions

Instrument: Agilent 1200 HPLC system with UV detector.

Computer: An HP Compaq system connected to HP Laser Jet 4050 Printer

Column: Inertsil C 8 (4.6 mm X 150 mm, 5µm).

λ = 215 nm

Column temperature: Ambient

Mobile phase: Methanol: Buffer *(3: 100). Isocratic condition

Flow rate: 1.5 mL/ min.

Injection volume: 20.0 µL

*The buffer is a quaternary mixture in proportion of 7:6:1:100 of 10N NaOH: 80% phosphoric acid: Triethylamine: Water.

Following is an illustration of percent recovery calculation involving the ORA method and the Quick & Easy method of Pilocarpine hydrochloride analysis performed very recently in our laboratory.

Table 1: HPLC Chromatographic data from figures A to H

Sample			**Area***
A_U Spike	Figure G	(1:1 mixture of standard + sample)	879.786
A_U Spike duplicate	Figure H	(1:1 mixture of standard + sample)	877.182
A_S Std.	Figure. A- E	(Average of 5 injections from HPLC system suitability)	907.555
A_U Sam.	Figure F	(Assay)	858.659

*** Area values from Tables 2 and 3**

ORA METHOD

$$X \text{ (assay)} = \frac{\text{(Spike sample area) (Std. wt x purity) (Spike volume)}}{\text{(Std. area) (Std. volume)}}$$

$$\text{Spike 1} = \frac{(879.786)\ (10.77 \text{ mg} \times 0.998)\ (2.0\ \cancel{\text{mL}})}{(907.555)\ (25.0\ \cancel{\text{mL}})\ (25.0\ \cancel{\text{mL}}/\ (3.0\ \cancel{\text{mL}})}$$

$$= 0.10002 \text{ mg}$$

$$\text{Spike 2 (duplicate)} = \frac{(877.182)\ (10.77 \text{ mg} \times 0.998)\ (2.0\ \cancel{\text{mL}})}{(907.555)\ (25.0\ \cancel{\text{mL}})\ (25.0\ \cancel{\text{mL}}\ /\ (3.0\ \cancel{\text{mL}})}$$

$$= 0.09973 \text{ mg}$$

K is the sum of the sample concentration and standard concentration in the spike solution which is calculated from the assay of the unknown sample and the concentration of standard is derived from its preparation.

K = (Wt. of sample + Wt. of standard)

$$\text{Wt. of sample: mg in 1.0 mL} = \frac{\text{(Sample area) (Std wt.)(Purity) x (1.0 mL)}}{\text{(Std. area) Std. volume)}}$$

$$= \frac{(858.659)\ (10.77 \text{ mg})\ (0.998) \text{x}\ (1.0\ \cancel{\text{mL}})}{(907.555)\ (25.0\ \cancel{\text{mL}})\ (25.0\ \cancel{\text{mL}}/\ (3.0\ \cancel{\text{mL}})}$$

$$= 0.04881 \text{ mg}$$

$$\text{Wt. of standard in the working standard solution: mg in 1.0 mL} = \frac{\text{(Std. wt x Purity) x (1.0 mL)}}{\text{(Std. volume)}}$$

$$\text{Concentration of the working standard solution} = \frac{(10.77 \text{ mg})\ (0.998)}{(208.33\ \cancel{\text{mL}})} \text{ x } (1.0\ \cancel{\text{mL}})$$

$$= 0.05159 \text{ mg}$$

K = 0.04881 mg + 0.05159 mg = 0.10040 mg

% Spike Recovery: = $\frac{X}{K}$ **(100)**

$$\text{Spike 1} = \frac{0.10002\ \cancel{\text{mg}}\ (100)}{0.10040\ \cancel{\text{mg}}} = 99.63\ \% = R_1$$

Spike 2(duplicate) = $\frac{0.09973 \text{ ~~mg~~} (100)}{0.10040 \text{ ~~mg~~}} = 99.33\ \% = R_2$

RPD = $\frac{(R_1 - R_2)\ (100)}{R}$ where R = average of R1 and R_2

= $\frac{(99.63 - 99.33)\ (100)}{99.48} = 0.3\ \%$

RPD = 0.3 %

QUICK AND EASY METHOD (*Pal's formula*) [2, 3]

$$\% \text{ Spike Recovery} = \frac{(\text{Spike solution area})\ (100)}{½\ (\text{Sample area} + \text{Average standard area})} \quad (2)$$

Or

$$\% \text{ Spike Recovery} = \frac{A_{U\ Spike}\ (100)}{½\ (A_{U\ Sam.} + A_{Std.})}$$

Spike 1 = $\frac{(879.786)\ (100)}{½\ (858.659 + 907.555)}$

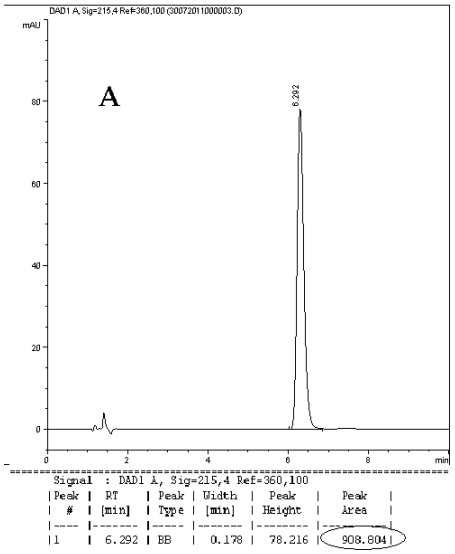
= 99.62 %

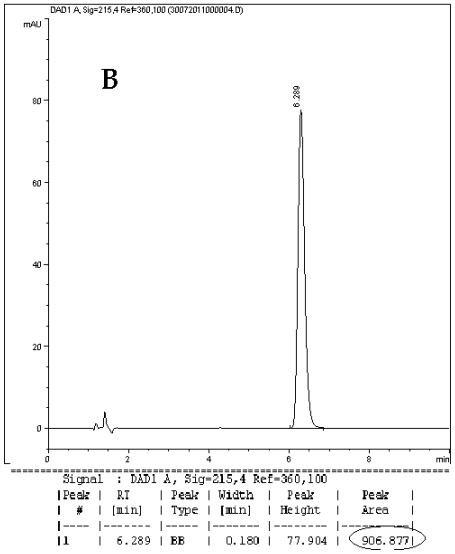
Spike 2 (duplicate) = $\frac{(877.182)\ (100)}{½\ (858.659 + 907.555)}$

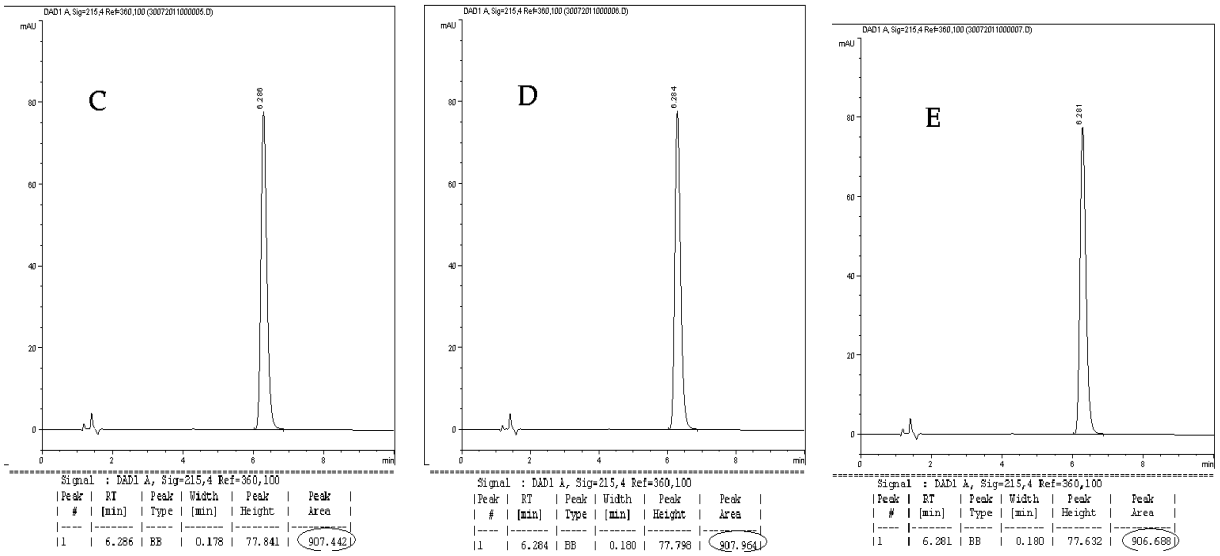
= 99.32 %

RPD = $\frac{(99.62 - 99.32)\ (100)}{99.47}$

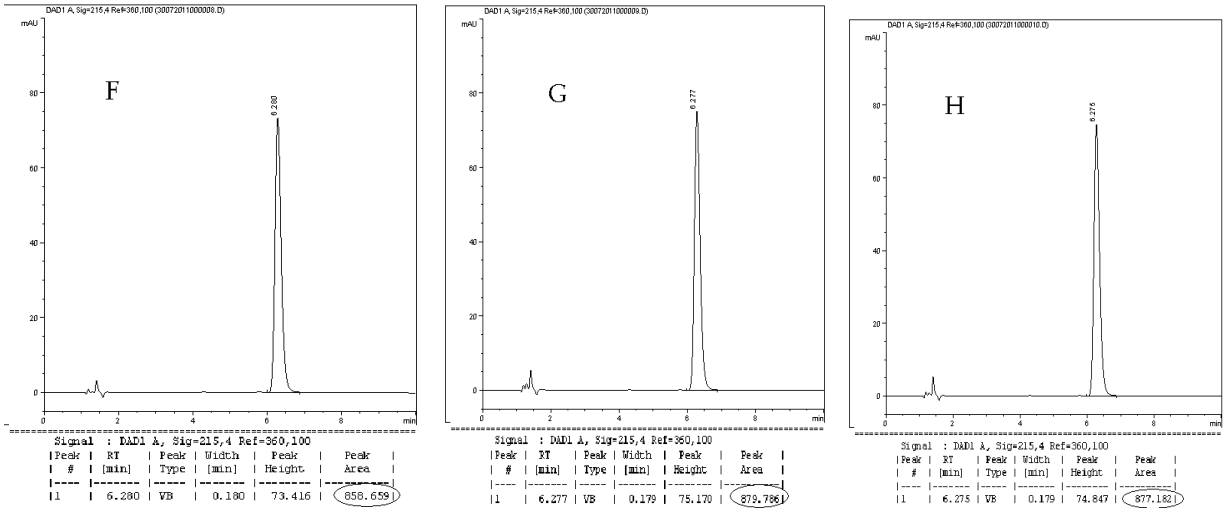
= 0.3%







Figures A, B, C, D, and E: HPLC profiles of Pilocarpine hydrochloride reference standard injections demonstrate the reproducibility and system suitability performance. Data presented underneath each figure corresponds to the original peak areas generated by HPLC analysis of reference standard.



Figures G to H: HPLC profiles of sample and two spiked samples and original peak area values are presented underneath each figure.

Table 2: Pilocarpine hydrochloride peak areas and the % RSD from figures A to E.

Figure	Reference std. area $A_{Std.}$
A	908.804
B	906.877
C	907.442
D	907.964
E	906.688
Average	**907.555**
%RSD	**0.095%**

Table 3: HPLC Data for Pilocarpine hydrochloride

Figure 1	Area A_U
F Sample $A_{U\ Sam.}$ (Assay)	858.659
G Spike $A_{U\ Spike}$	879.786
H Spike duplicate $A_{U\ Spike}$	877.182

RESULTS AND DISCUSSION

Results obtained using the two methods as illustrated above produced identical RPD values = 0.3 % of spike recoveries. Table 4 shown below, compares results of % spike recoveries in duplicate and the corresponding RPD values obtained by both the ORA and the Quick and Easy methods for several drug analyses performed in our laboratory. No appreciable differences in the % recovery and the RPD values were noted between the two methods involving any of the data presented in Table 4.

Table 4 Comparison of percent spike calculations using Quick and Easy method and ORA method

Analyte	Parameters in equations 1 &2	Values	Quick and Easy	ORA
1. Tylenol	Spike 1 (area)	303.088	100.13	100.16
	Spike 2 (area)	302.918	100.07	100.10
	Std area Average	390.428		
	Sample area	214.983		
	Sample amount in spike mg / mL	0.011528		
	Std Conc. mg / mL	0.010477		
	Spike vol. mL	4		
	Std amount in spike mg / mL	0.020955		
	Total drug in spike mg	0.032483		
	RPD		**0.06**	**0.06**
2.Ibuprofen	Spike 1	3473.785	98.64	98.63
	Spike 1 Int. std	4766.099		
	Spike 2	3486.133	98.62	98.64
	Spike 2 Int. std	4782.372		
	Std area Average	3604.313		
	Int. Std area Average	4806.094		
	Sample area	3463.1		
	Int. std sample	4756.457		
	Std Conc. mg / mL	12.08457		
	Sample amount in spike mg / mL	11.732		
	Spike vol. mL	2		
	Total drug in spike mg	23.81657		
	RPD		**0.02**	**0.01**
3. Acetaminophen	Spike 1	689.987	100.01	100.00
	Int. std	958.329		
	Spike 2	682.886	100.04	100.03
	Int. std	948.173		

	Std area Average	658.369		
	Int. Std area Average	943.938		
	Sample area	702.237		
	Int. std sample	945.985		
	Std Conc. mg / mL	0.10205		
	Std added	0.20410		
	Sample amount in spike mg / mL	0.21726		
	Spike vol. mL	4		
	Total drug in spike mg	0.42136		
	RPD		**0.03**	**0.03**
4. Caffeine	Spike 1	624.4763	103.40	103.40
	Spike 2	624.3365	103.40	103.37
	Std area Average	640.968		
	Sample area	566.9232		
	Sample amount in spike mg / mL	0.019458		
	Std Conc. mg / mL	0.022		
	Spike vol. mL	2		
	Total drug in spike mg	0.041458		
	RPD		**0**	**0.03**
5.Miconazole nitrate	Parameters in equations 1 & 2	Values	Quick & Easy	ORA
	Spike 1	7418.458	100.48	100.48
	Spike 2	7418.153	100.48	100.48
	Std area Average	7134.719		
	Sample area	7630.657		
	Sample amount in spike mg / mL	0.606729		
	Std Conc. mg / mL	0.567293		
	Spike volume mL	2		
	Total drug in spike mg	1.174018		
	RPD		**0**	**0**
UV analysis				
6. Tylenol	Spike 1 Absorbance	0.601	100.59	100.58
	Spike 2 Absorbance	0.602	100.75	100.75
	Std Absorbance	0.624		
	Sample Absorbance	0.571		
	Sample amount in spike mg / mL	9.48771		
	Std Conc. mg / mL	10.36836		
	Spike volume mL	2		
	Std amount in spike mg	19.85607		
	RPD		**0.09**	**0.08**

Note: For non violative finished products (capsules, tablets), the % recovery will always be between 90-110 % of label claim and 98-102 % for Active Pharmaceutical Ingredients

Mathematically, both ORA and the Quick & Easy method are derived using similar logic, but the former is quite a round about method and involves several terms: three in the numerator for determining the *X* value and two in the denominator for determining the *K* value. To arrive at

the final step in calculating the percent spike recovery where proper values are substituted for *X* and *K* in equation (1): % Recovery = *X/K* (100), quite a bit of time and effort is spent at the desk. Quick & Easy method is very simple and straightforward. The two areas, A_U are generated by HPLC injections of the two individual spiked solution of 1+1 mixture of the working standard and the working sample-assay solution. These values, 879.786 and 877.182 are shown in Figs. G and H and tabulated in Table 3. These peak areas are the only unknown response areas that are to be determined experimentally and are used in the numerator of equation (2). The two numbers in the denominator are readily available: 907.555, the average response area of the standard solution obtained during the system suitability experiments, Figs. A through E shown in Table 2. The other number in the denominator is the data obtained from the assay of the sample: the sample response area 858.659, Fig. F, Table 3. The coefficient ½ in the denominator is the dilution factor produced by mixing two working solutions in equal proportion: 1.0 mL standard solution and 1.0 mL sample assay solution. The spike mixture results in half the amount of solutes in a total of 2.0 mL. The denominator is the same number in the two spike recovery calculations. It can be deemed as the average of the two response areas in the denominator, *only* when it is a 1:1 mixture of standard and sample solutions.

When the spike mixture is made of different proportions other than 1:1, for example: 1:2 or 1:3 of the standard and sample, the dilution factor will have to be changed accordingly to reflect the variation in the concentration of the spike mixture and hence the coefficient in the denominator of the formula will have to be adjusted to 1/3 or 1/4 respectively. The 1:1 proportion is preferred because it is expedient and more importantly will satisfy the linearity requirement, when the concentration of the sample and standard solutions are close.

Calculations involving the Quick & Easy method are so simple that the recoveries can be quickly estimated as soon as the HPLC data pops up on the screen of the instrument. In the ORA method, the numerator *X* and the denominator *K* are weights in milligrams, as shown in the illustration above. It is important to note that there are no weights involved in the calculations using the Quick & Easy method formula. All the terms used in the equation (2) are response areas or UV absorbance obtained straight from the instrument data.

CONCLUSION

As the name implies, Quick & Easy method is a very simple, efficient and convenient way of determining percent spike recoveries and the associated RPD values of duplicate spike solutions to evaluate precision in drug analyses by HPLC, GC or UV methods. The simplicity of the

method saves time and effort when compared to the ORA method.

ACKNOWLEDGEMENT

Jim Stuart, Chemist PRL-NW.

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