Review article

Incorrect determination of lower limit of quantification (LLOQ) of analytical methods

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SUMMARY

Objectives: Generally, a new analytical method for publication in a scientific journal should report some parameters to claim that the technique has acceptable validity and sensitivity in contrast to other methods. The most commonly reported parameters are limit of detection (LOD), limit of quantification (LOQ), and lower limit of quantification (LLOQ). Recently, LLOQ was selected by researchers to show the lowest concentration level that can be determined. Based on the FDA's guidelines, some criteria should be considered in reporting LLOQ. **Methods:** In this study, previously reported analytical methods were investigated to check if they considered FDA's criteria (relative standard deviation of LLOQ, signal-to-noise, and back-calculated error for LLOQ). Besides the FDA guideline's criteria, the established criteria based on the calibration curve equation and the linear range were calculated to confirm the validity of the reported LLOQ. **Results:** Analysis of data indicates that the most LLOQ values are not defined based on FDA guidelines, and they do not show the correct sensitivity value of the reported analytical method. **Conclusions:** Considering the all of the recommended criteria for LLOQ are necessary to correct determination of sensitivity and linear range of analytical methods.

Keywords: Analytical method; FDA; LLOQ; LOD; LOQ

RESUMEN

Determinación incorrecta del límite inferior de cuantificación (LIDC) de los métodos analíticos

Objetivos: Generalmente, un nuevo método analítico para publicación en una revista científica debe reportar algunos parámetros para afirmar que la técnica tiene una validez y sensibilidad aceptables en contraste con otros métodos. Los parámetros informados con mayor frecuencia son el límite de detección (LDD), el límite de cuantificación (LDC) y el límite inferior de cuantificación (LIDC). Recientemente, los investigadores seleccionaron LIDC para mostrar el nivel de concentración más bajo que se puede determinar. Según las pautas de la FDA, se deben tener en cuenta algunos criterios al informar el LIDC. **Métodos:** En este estudio, se investigaron los métodos analíticos informados previamente para verificar si consideraban los criterios de la FDA (desviación estándar relativa del LIDC, relación señal-ruido y error retrocalculado para el LIDC). Además de los criterios de las directrices de la FDA, se calcularon los criterios establecidos basados en la ecuación de la curva de calibración y el rango lineal para

confirmar la validez del LIDC informado. **Resultados:** El análisis de datos indica que la mayoría de los valores LIDC no están definidos según las pautas de la FDA y no muestran el valor de sensibilidad correcto del método analítico informado. **Conclusiones:** Considerando que todos los criterios recomendados para LIDC son necesarios para la correcta determinación de la sensibilidad y rango lineal de los métodos analíticos.

Palabras clave: Método analítico; Administración de Alimentos y Medicamentos; LIDC; LDD; LDC

RESUMO

Determinação incorreta do limite inferior de quantificação (LIDQ) dos métodos analíticos

Objetivos: Geralmente, um novo método analítico para publicação em um periódico científico deve relatar alguns parâmetros para afirmar que a técnica tem validade e sensibilidade aceitável em contraste com outros métodos. Os parâmetros mais comumente relatados são limite de detecção (LDD), limite de quantificação (LDQ) e limite inferior de quantificação (LIDQ). Recentemente, o LIDQ foi selecionado por pesquisadores para mostrar o menor nível de concentração que pode ser determinado. Com base nas diretrizes do FDA, alguns critérios devem ser considerados ao relatar o LIDQ. **Métodos:** Neste estudo, métodos analíticos relatados anteriormente foram investigados para verificar se eles consideravam os critérios do FDA (desvio padrão relativo do LIDQ, relação sinal-ruído e erro retrocalculado para LIDQ). Além dos critérios da diretriz da FDA, os critérios estabelecidos com base na equação da curva de calibração e na faixa linear foram calculados para confirmar a validade do LIDQ relatado. **Resultados:** A análise dos dados indica que a maioria dos valores de LIDQ não são definidos com base nas diretrizes do FDA e não mostram o valor de sensibilidade correto do método analítico relatado. **Conclusões:** Considerando todos os critérios recomendados para LIDQ, é necessário determinar corretamente a sensibilidade e a faixa linear dos métodos analíticos.

Palavras-chave: Método analítico; FDA; LIDQ; LDD; LDQ

1. INTRODUCTION

The lowest concentration level that can be reliably quantified is the most common parameter of an analytical method for. In the most of published articles, this value was compared with previous analytical techniques to confirm better sensitivity. Limit of detection (LOD), limit of quantification (LOQ) [1] and lower limit of quantification (LLOQ) [2, 3] are currently used in determining the sensitivity of the analytical method and calculated based on the calibration curve.

LOD and LOQ are classic parameters to determine the minimum concentration of an analyte that can be detected and quantified, respectively. Generally, three and ten folds of standard deviation divided by slope were used for the determination of LOD and LOQ, respectively. However, various methods were proposed for the calculation of standard deviation of an analytical method i.e. standard deviations of the blank, calibration curve and intercept. Therefore, significantly different numerical values of LOD and LOQ are obtained for the same analytical method [4, 5].

FDA proposed an alternative parameter for LOD and LOQ which is named LLOQ. LLOQ looks like is simple parameter and it is the lower limit of a calibration curve. Especially in the established method based on spectroscopy techniques and complex matrix e.g. biological samples, LLOQ was proposed as an alternative parameter [4]. However, the correct determination of LLOQ is a challenging issue. It is suggested by the FDA and the method for its determination has some criteria which are described in the method section.

Based on previous studies, some of the reported calibration ranges were not determined correctly by researchers and a new parameter which named "ratio" for validity evaluation of analytical was proposed by our group. Incorrect determination of calibration curve gives false LLOQ value and application of established analytical method for quantification of an analyte is not correct [6].

In this study, published articles which "LLOQ" was available in the abstract of the articles were chosen from the Scopus database. The articles which have no appropriate information were excluded. Then, the selected article was investigated from the viewpoint of considering LLOQ criteria and the results have been discussed. In addition, "ratio" as a main parameter to check the validity of the analytical method was reported.

2. METHODS

The Scopus database was used for collecting articles. LLOQ in the abstract of published articles in 2021 was selected as a keyword. In the first step, reported LLOQ, linear range, and linear regression equation data of reported analytical methods were collected and listed in Table 1. From selected articles, 185 analytical methods from 66 articles that reported all of the data were recorded. In the next step, we checked if the LLOQs had been reported under the required condition or not. Determination criteria for LLOQ were evaluated based on FDA's guidelines as follows [7]:

1. Signal/noise=5
2. The relative standard deviation (RSD) of LLOQ should be less than 20%.
3. The back-calculated error for LLOQ should be less than 20%.

In addition, the reported parameter for evaluation accuracy of linear range based on the upper limit of the calibration curve (ULOQ) and LLOQ which was reported in previous work, was calculated as follows:

$$\text{Ratio} = \frac{(\text{calculated response for ULOQ/calculated response for LLOQ})}{(\text{ULLOQ/LLOQ})}$$

In an ideal situation, the ratio should be 1; the ratio between 0.5 and 1.5 was considered correct and valid LLOQ and linear range [6].

3. RESULTS AND DISCUSSION

Table 1 lists details of studied analytical methods for evaluating the applied technique for the determination of LLOQ. Generally, the lower limit of the calibration curve in all articles was declared as LLOQ without the details of the FDA's criteria. Signal/noise=5 and RSD value for LLOQ < 20% are the main criteria which did not mention in the determination of LLOQ. The calculated "ratio" of each method indicates that only 54% of methods have a numerical value between 0.5-1.5. This issue confirms the importance of all criteria for LLOQ determination in developing analytical methods and this issue should be considered by editors and reviewers of journals. A considerable issue is observed in the obtained ratios for the developed methods for the analysis of dexamethasone in various matrices (2, 1.27 and 1.63 in plasma, retina, and vitreous, respectively). It confirms the importance of the matrix effect in developing an analytical method.

In some reported cases in international journals, lower limit of calibration curve based R^2 value was determined as LLOQ while the intercept value of calibration curve is high [4] and

give negative values for back-calculated concentration (Figure 1). In addition, a bar chart instead of a scatter plot was applied for plotting a calibration curve while in a bar chart continuous values were inserted in the X-axis [8]. In some cases, the logarithm of concentration was used for plotting a calibration curve without considering back-calculated concentration based on the regression equation of calibration [9]. These errors in developing an analytical method and determination of linear range show the importance of reporting data of calibration curve and considering FDA criteria in reporting an analytical method. Various methods were applied for sensitivity determination of an analytical method, and as we highlighted in this article, the principal criteria were not considered in reporting LLOQ. In some studies, only one criterion i.e., 5-fold signal-to-noise ratio was applied to the calculation of LLOQ. In these articles, LLOQ values are exactly half of LOQ [10]. Surprisingly, in most articles, the analytical methods are compared together without considering these issues. Defined "ratio" in previous work which applied in this study is a valuable technique for evaluating the accuracy of reported linear range. In addition, alternative statistical methods such as double logarithm of concentration and response which were proposed by Zhu [11] could be applied by researchers for extending linear range of an analytical method.

Table 1. Details of collected analytical methods, including linear ranges (ULOQ and LLOQ), calibration curve equations, and numerical values of the defined ratio.

Code	Analyte	LLOQ	ULOQ	unit	Slope	Intercept	Ref.	Ratio
1	10-O-vanilloylaucubin	2.5	250	ng/mL	0.00126	0.00521	[12]	0.38
2	11-deoxycortisol	1.3	250	ng/mL	0.15	-0.49	[13]	-0.65
3	11-hydroxy-Delta(9)-tetrahydrocannabinol	0.2	100	ng/mL	0.0293	0.0001	[14]	0.98
4	11-keto-β-boswellic acid	7.50	1500.00	ng/mL	0.0284	-0.3621	[15]	-1.42
5	17-hydroxyprogesterone	1.2	250	ng/mL	0.10	-0.33	[13]	-0.56
6	21-deoxycortisol	0.19	250	ng/mL	0.15	-0.46	[13]	-0.07
7	3-acetyl-11-keto-β -boswellic acid	7.50	1500.00	ng/mL	0.1104	0.0967	[15]	0.90
8	6-Hydroxydexamethasone	0.1	100	ng/mL	0.512	-0.00307	[16]	1.06
9	Acetaminophen	10	500	ng/mL	0.00212	0.00521	[17]	0.81
10	Aconine	0.10	40.00	ng/mL	0.00322	0.00119	[15]	0.21
11	Adenosine diphosphate	0.1	20	μg/mL	14.041	3.0471	[18]	0.32
12	Adenosine monophosphate	0.1	20	μg/mL	28.864	7.0041	[18]	0.30
13	Adenosine triphosphate	0.1	20	μg/mL	8.9019	1.6839	[18]	0.35
14	Agnuside	1	500	ng/mL	0.025	0.00614	[12]	0.80
15	Alanine	0.50	4120	ng/mL	140.2096	746.8086	[19]	0.09
16	Aldosterone	15	5000	pg/ml	1.088	-0.114	[20]	1.01
17	Aloperine	5	2000	ng/mL	0.46	0.0029	[21]	1.00
18	Ampicillin	0.25	200	μg/mL	2.103	5.2332	[22]	0.09
19	Androstenedione	0.18	250	ng/mL	0.10	-0.29	[13]	-0.07
20	Angiotensin II	15	5000	pg/ml	0.896	-1.005	[20]	1.08
21	Arginine	1.94	3969	ng/mL	33.1698	293.494	[19]	0.18
22	Aristolochic acids I, mouse kidney	5.24	400	ng/mL	0.031	-0.065	[23]	1.66
23	Aristolochic acids I, mouse liver	4.62	400	ng/mL	0.028	-0.065	[23]	2.00

24	Aristolochic acids I, mouse serum	4.82	500	ng/mL	0.026	0.015	[23]	0.89
25	AS-358	1.02	500	ng/mL	0.00313	-0.0000453	[24]	1.01
26	Asenapine Maleate	0.1	14	µg/mL	36384	-656.84	[25]	1.22
27	Asparagine	2.46	5040	ng/mL	14.1428	27.9812	[19]	0.55
28	Aspartic acid	42.19	5400	ng/mL	4.9136	30.8616	[19]	0.87
29	Atractylenolide I	0.44	400.5	ng/mL	0.00290	-0.00119	[26]	14.82
30	Atractylenolide III	0.54	490.5	ng/mL	0.00439	0.22385	[26]	0.01
31	Atrovastatin	0.011	50.000	ng/patch	78710	6966	[27]	0.11
32	Avapritinib	2	4000	ng/mL	0.332162	0.132643	[28]	0.83
33	AYPTPLR-1	25	5000	mIU/mL	2822.08864	22166.46211	[29]	0.76
34	AYPTPLR-2	25	5000	mIU/mL	2326.43121	18774.36376	[29]	0.76
35	AYPTPLR-3	25	5000	mIU/mL	505.05598	6483.40981	[29]	0.66
36	Bezafibrate	0.022	50.000	ng/patch	120600	1737	[27]	0.60
37	Borneol	2	5000	ng/mL	0.0003	0.0005	[30]	0.55
38	Bornyl acetate	2	1000	ng/mL	0.0002	0.0024	[30]	0.14
39	Budesonide	0.05	10.00	µg/ ml	162376	-943.55	[31]	1.13
40	Budesonide	0.05	5.00	µg/ ml	165522	89.816	[31]	0.99
41	Buprenorphine	5	100	pg/µL	0.1249	-0.0517	[32]	1.09
42	Calycanthine	1.0	200	ng/mL	0.001748	-0.00763	[33]	-0.29
43	Camphor	2	10000	ng/mL	0.0005	0.0118	[30]	0.08
44	Cannabidiol	0.2	100	ng/mL	0.0420	-0.0018	[14]	1.27
45	Cannabinol	0.2	100	ng/mL	0.0973	-0.0016	[14]	1.09
46	Caryophyllene Oxide	2	5000	ng/mL	0.0001	0.0067	[30]	0.03
47	Casticin	0.5	50	ng/mL	0.00168	-0.000216	[12]	1.34
48	Cedazuridine	1	500	ng/mL	0.0061	0.1313	[34]	0.05
49	Chlorambucil	0.075	15.0	µg/mL	0.15501	0.00244	[35]	0.83
50	Ciprofibrate	0.032	50.000	ng/patch	29660	281.6	[27]	0.77
51	cis-4-GG-crocin	10.00	3200.00	ng/mL	0.000247568	0.000353532	[36]	0.88
52	Clofibrate	0.050	50.000	ng/patch	14830	2477	[27]	0.23
53	Codein	5	100	pg/µL	0.1316	-0.0790	[32]	1.13
54	Cortisol	0.49	250	ng/mL	0.12	-0.11	[13]	-1.14
55	Cortisol-D4	71.7	44017.8	pg/ml	0.00309	-0.1088	[37]	1.96
56	Cortisone-13C3	61.9	75555.2	pg/ml	0.00274	0.00552	[37]	0.97
57	Cotinine	6.00	336	ng/mL	0.00335	0.00283	[38]	0.88
58	Curzerene	10.16	325.0	ng/mL	0.0747	-0.0064	[39]	1.01
59	Cyanide, aqueous solution	0.05	5	µg/mL	1.6393	-0.231	[40]	-0.53
60	Cyanide, blood solution	0.1	5	µg/mL	0.698	-0.0331	[40]	1.88
61	Cysteine	2.21	4519	ng/mL	13.0453	-14.8464	[19]	2.06
62	D4-cystine	5	5000	ng/mL	0.000974	0.00364	[41]	0.57
63	DA-7010 (novel antibacterial candidate)	10	10000	ng/mL	1.5989	0.0002938	[42]	1.00
64	Decitabine	1	500	ng/mL	0.0053	0.0915	[34]	0.06
65	Dehydro Amlodipine	0.24	24.58	nM	0.11176	0.03085	[43]	0.47
66	Dehydro Amlodipine	0.24	24.58	nM	0.1528	0.3293	[43]	0.11
67	Dehydroepiandrosterone	4.6	250	ng/mL	0.02	0.00	[13]	1.00
68	Dexametasone	1.5	150	ng/mL	0.0044	0.0015	[44]	0.82

69	Dexamethasone, plasma	0.1	100	ng/mL	0.414	-0.0207	[16]	2.00
70	Dexamethasone, retina	0.1	100	ng/mL	0.474	-0.00997	[16]	1.27
71	Dexamethasone, vitreous	0.1	100	ng/mL	0.560	-0.0216	[16]	1.63
72	Diazepam	5	100	pg/μL	0.1539	-0.0716	[32]	1.10
73	Dimethyltryptamine	3	1109	pg/mL	281.50213	0.00231	[45]	1.00
74	Diosgenin	0.50	447.00	ng/mL	0.0002563	0.00142	[26]	0.08
75	Diquat	0.05	20	μg/mL	316000.00	28200.00	[46]	0.36
76	Doxorubicin	0.017	8.6	μM	0.0365	0.533	[47]	0.00
77	ELVYETVR-2	25	5000	mIU/mL	2316.50438	18612.30584	[29]	0.76
78	Erdafitinib	0.05	2.00	μg/mL	0.9597	-0.0013	[48]	1.03
79	Eucalyptol	1	10000	ng/mL	0.0003	0.0022	[30]	0.12
80	Eugenol	1	1000	ng/mL	6.00E-05	-0.0009	[30]	-0.07
81	Ezetimibe	0.050	50.000	ng/patch	33540	537.1	[27]	0.76
82	Favipiravir	3	60.0	μg/mL	0.00003381	0.004900433	[49]	0.07
83	Fenofibrate	0.003	50.000	ng/patch	444900	341000	[27]	0.00
84	Fentanyl	1	100	pg/μL	0.0944	-0.0418	[32]	1.79
85	Fludrocortisone	10	1000	pg/ml	0.00191951	0.222242	[50]	0.09
86	Flurbiprofen	5	5000	ng/mL	0.0000082061	-0.000017079	[51]	1.71
87	Fluvastatin	0.018	50.000	ng/patch	42820	131.3	[27]	0.85
88	Galgravin	1.0	500	ng/mL	0.020756	0.005814	[52]	0.78
89	Gemfibrozil	0.017	50.000	ng/patch	10650	344.5	[27]	0.34
90	Ginsenoside Rb1	5	10000	ng/mL	0.0001	0.0005	[27]	0.50
91	Ginsenoside Rc	5	10000	ng/mL	0.0002	0.0010	[27]	0.50
92	Ginsenoside Rd	5	10000	ng/mL	0.0005	0.0028	[27]	0.47
93	Ginsenoside Rg1	0.63	389	ng/mL	0.00002525	0.00009435	[26]	0.15
94	Ginsenoside Rh1	5	10000	ng/mL	0.0006	0.0015	[53]	0.67
95	Globotriaosylsphingosine	0.25	100	ng/mL	0.42302	0.14881	[54]	0.42
96	Glutamic acid	2.79	5720	ng/mL	21.7154	57.0464	[19]	0.52
97	Glutamine	4.96	5080	ng/mL	25.9211	4.1991	[19]	0.97
98	Glycine	16.25	4160	ng/mL	6.5835	17.8001	[19]	0.86
99	HER-2 protein	0.000001	1	nM	-45.233	36.633	[55]	0.00
100	Histamin	0.01	20	ng/mL	0.97	0.031	[56]	0.24
101	Histamin	0.1	32	pg/mL	1.11	-0.029	[56]	1.35
102	Histidine	4.25	4352	ng/mL	39.1237	302.7673	[19]	0.36
103	Hydrocortisone Phosphate	5	1000	ng/mL	0.043	0.054	[57]	0.80
104	Hydroxycotinine	6.00	336	ng/mL	0.01801	0.01541	[38]	0.88
105	Hydroxyproline	8.05	4120	ng/mL	33.6225	9.4106	[19]	0.97
106	Isoleucine	0.61	5000	ng/mL	71.1636	111.2613	[19]	0.28
107	Isoliquiritigenin	0.39	356.10	ng/mL	0.00220	-0.0002625	[26]	1.44
108	Letrozole	0.01	1	μg/ ml	3583.7	734.63	[58]	0.06
109	Leucine	1.00	4080	ng/mL	81.4774	135.3090	[19]	0.38
110	Linezolid	3.0	25	mg/mL	0.0008	-0.0012	[59]	1.88
111	Lipoprotein lipase peptide 1	12.5	2500	pM	0.000928	0.0114	[60]	0.51
112	Lorazepam	5	100	pg/μL	0.0172	0.0285	[32]	0.76
113	Lovastatin	0.015	50.000	ng/patch	61880	2088	[27]	0.31

114	luteolin	0.2	50	ng/mL	0.00448	0.000939	[12]	0.49
115	Lysine	3.44	3521	ng/mL	32.6282	198.7964	[19]	0.36
116	Lysosomal acid lipase	25	2500	pM	0.00158	-0.0163	[60]	1.70
117	Malondialdehyde	0.2	20	μg/g	21.749	-8928.3	[61]	0.01
118	Melatonin	1	1000	pg/ml	0.00774	-0.00079	[62]	1.11
119	Methionine	1.11	4560	ng/mL	55.3991	-26.8628	[19]	1.78
120	Methotrexate	10	500	ng/mL	0.000224	0.000654	[17]	0.78
121	Methoxyfuranodiene	7.88	252.0	ng/mL	0.0363	-0.0127	[39]	1.05
122	Methylprednisolone	10	500	ng/mL	0.0296	0.4	[17]	0.44
123	Myrislignan	1	1000	ng/mL	0.00202	-0.00104	[63]	2.06
124	Neferine	0.31	279.00	ng/mL	0.00133	0.00776	[26]	0.05
125	Neoline	0.50	200.00	ng/mL	0.0202	0.0118	[15]	0.46
126	Nicotine	0.50	28.0	ng/mL	0.15171	0.03676	[38]	0.68
127	Nicotinic Acid	0.012	50.000	ng/patch	63740	116500	[27]	0.01
128	Nordiazepam	1	100	pg/μL	0.2284	-0.2942	[32]	-3.43
129	Nuciferine	0.41	367.50	ng/mL	0.02406	-0.00469	[26]	1.91
130	Omeprazole	1	500	ng/mL	0.00725	0.00028	[17]	0.96
131	Oxazepam	10	100	pg/μL	0.1000	-0.0004	[32]	1.00
132	Pachymic acid	0.38	355.50	ng/mL	0.000076916	0.00537	[26]	0.01
133	Palmatine	1	500	ng/mL	0.015	-0.021	[64]	-2.49
134	Panaxadiol	0.44	397.50	ng/mL	0.00102	0.00061385	[26]	0.42
135	Paraquat	0.05	20	μg/mL	376000.00	133.00	[46]	0.99
136	p-Coumaric Acid	0.2	20	ng/mL	6819.89	1013.91	[65]	0.58
137	p-Cresol	0.5	30	μg/mL	8.9645	1.89	[66]	0.71
138	Phenylalanine	1.21	4960	ng/mL	293.9884	260.6369	[19]	0.58
139	Picrocrocin	10.00	3200.00	ng/mL	0.002674921	0.00435742	[36]	0.86
140	Platycodin D	0.42	382.50	ng/mL	0.000035807	0.0000274	[26]	0.36
141	Pravastatin	0.046	50.000	ng/patch	47100	5685	[27]	0.28
142	Pregnenolone	6.4	250	ng/mL	0.002	0.02	[13]	0.41
143	Proline	2.05	4200	ng/mL	246.6109	2569.574	[19]	0.16
144	Propofol glucuronide	0.5	500	μg/mL	0.4545	3.1022	[67]	0.07
145	Propranolol	5	100	pg/μL	0.1148	-0.0418	[32]	1.07
146	Putative phospholipase B-like 2 peptide 7	6.25	2500	pM	0.0017	-0.0053	[60]	1.99
147	R-(+)-Verapamil	0.5	500	ng/mL	0.0025	0.0389	[68]	0.03
148	R-Dioxopromethazine	1.00	80.00	ng/mL	0.079	0.034	[69]	0.70
149	Remogliflozin etabonate	5	300	ng/mL	0.0144	0.0307	[70]	0.71
150	Renin	0.1	33	pg/ml	0.856	0.00129	[20]	0.99
151	Rosuvastatin	0.013	50.000	ng/patch	45620	739.8	[27]	0.45
152	S-(-)-Verapamil	0.5	500	ng/mL	0.0023	0.0434	[68]	0.03
153	Safranal	10.00	3200.00	ng/mL	0.007429112	0.02994744	[36]	0.71
154	Sarecycline	1	1000	ng/mL	0.00197147	-0.0015151	[71]	4.32
155	S-Dioxopromethazine	1.00	80.00	ng/mL	0.079	0.037	[69]	0.69
156	Serine	4.49	4600	ng/mL	36.0282	135.8935	[19]	0.54
157	Simvastatin	0.007	50.000	ng/patch	133500	41680	[27]	0.02
158	SKBR-3 cells	20	2000	cell/mL	-0.3575	1021	[72]	0.00

159	Songorine	0.20	80.00	ng/mL	0.0146	0.00709	[15]	0.29
160	Sophoridine	2	2000	ng/mL	0.009214	0.007572	[73]	0.71
161	Sulbactam	0.25	200	μg/mL	0.0588	0.0008	[22]	0.95
162	THC	0.2	100	ng/mL	0.0484	-0.0011	[14]	1.13
163	THC-COOH	0.2	100	ng/mL	0.0616	-0.0016	[14]	1.15
164	Theophylline	10	500	ng/mL	0.000211	0.000867	[17]	0.71
165	Threonine	2.46	5040	ng/mL	29.4302	140.763	[19]	0.34
166	Trans crocetin	10.00	3200.00	ng/mL	0.002001102	0.002474666	[36]	0.89
167	Trans-2-gg-crocin	10.00	3200.00	ng/mL	0.002490268	-0.000108653	[36]	1.00
168	Trans-3-Gg-crocin	10.00	3200.00	ng/mL	0.002373485	-0.000003492705	[36]	1.00
169	Trans-4-GG-crocin	10.00	3200.00	ng/mL	0.001106907	0.000787308	[36]	0.93
170	Trelagliptin	4	1000	nM	0.0036	0.0099	[74]	0.59
171	Trifluralin	0.00001	1	mM	4.09	0.35	[75]	0.00
172	Tryptophan	1.19	4880	ng/mL	168.0951	-8.2806	[19]	1.04
173	Tyrosine	1.31	5360	ng/mL	65.7911	47.0506	[19]	0.65
174	Upadacitinib	1.0	200	ng/mL	0.0754	0.1276	[76]	0.37
175	Valine	1.22	5000	ng/mL	410.7137	920.6263	[19]	0.35
176	Valproic Acid	0.075	15.0	μg/mL	0.32003	-0.02029	[35]	6.44
177	Vildagliptin	5	300	ng/mL	0.0118	-0.0014	[70]	1.02
178	VTVMGGFK-1	25	5000	mIU/mL	1351.99987	12817.41859	[29]	0.73
179	VTVMGGFK-3	25	5000	mIU/mL	458.53518	6670.91221	[29]	0.63
180	α-Pinene	3.97	127.0	ng/mL	0.0502	-0.011	[39]	1.06
181	α-Thujone	2	5000	ng/mL	0.0006	-0.0051	[30]	-0.31
182	β-Caryophyllene	2	10000	ng/mL	0.0003	0.0068	[30]	0.08
183	β-D-N4-hydroxycytidine	1	5000	pmol/sample	0.0153	-0.00124	[77]	1.09
184	β-D-N4-hydroxycytidine-triphosphate	1.00	1500	pmol/sample	0.0153	-0.00124	[77]	1.09
185	β-Elemene	21.38	684.0	ng/mL	0.2011	-0.1613	[39]	1.04

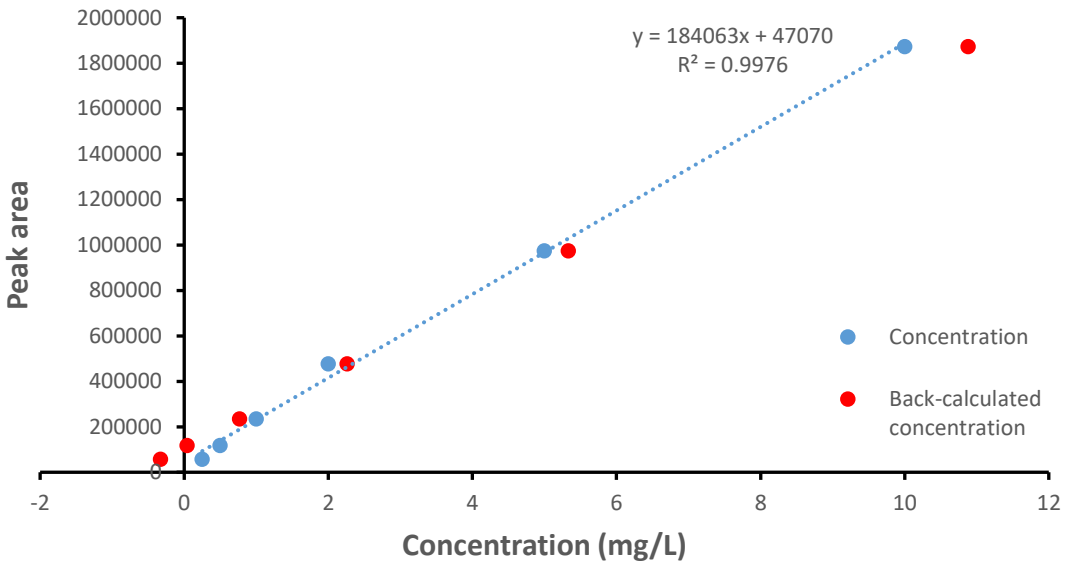

Figure 1. Peak area vs. concentration of an analytical method. Blue points are the concentrations of standard solution and red points are back-calculated concentrations. The R^2 value is higher than 0.99 while there is a significant difference between the concentrations of standard solution and back-calculated concentrations and the back-calculated LLOQ value is negative.

4. CONCLUSION

Developing an accurate method for quantification of an analyte is crucial. The most of decisions and guidelines in chemistry and medical sciences is performed based on the obtained concentration of an analyte. In medical sciences, decision about the efficacy of a therapeutic protocol is performed based on the concentration of drugs and biomarkers in biological samples. Therefore, this issue shows the importance of developing an accurate analytical method. LOD and LOQ which are the classic parameters for determination of the sensitivity of a method have some weaknesses discussed in previous works. LLOQ is recommended by FDA and, recently applied by researchers for evaluating the sensitivity of a method, thoroughly analyzed in this work. Some criteria were proposed for the determination of LLOQ and analysis of published articles indicates that the most of articles did not consider these parameters in reporting LLOQ. This issue confirms the importance of more attention by editors and journals in assessing manuscripts related to developing analytical methods.

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CONFLICTS OF INTEREST

Authors have no conflict of interest.

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